MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY NATIONAL AGRARIAN UNIVERSITY

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GUO LIPING

UDC 637.5.04/.07

DISSERTATION

FORMATION OF FUNCTIONAL AND TECHNOLOGICAL PROPERTIES OF PORK BY THE INFLUENCE OF VITAMIN D_3 ON THE DIFFERENTIAL MATERNAL LEVEL OF PIGS

Specialty 181 - Food Technology
Field of study - Food Science and Technology

Submitted for a scientific degree of doctor of philosophy
The dissertation contains the results of own research. The use of ideas, results and texts of other authors have references to the relevant source
_____Liping Guo

Guo Liping
数字签名由 Guo Liping
DN：C=CN, OU=Sumy national agrarian university, O=Sumy national agrarian university, CN=Guo Liping, E=529129326@qq.com
原因：我是该文档所有者
位置：
日期：2023.01.17 21:58:12+08'00'
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Scientific supervisor: Sergiy Melnychuk, Professor

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Sumy – 2022

ANNOTATION

Guo Liping Formation of functional and technological properties of pork by the influence of Vitamin D3 on the differential maternal level of pigs. - Qualifying scientific work on the rights of the manuscript.

The dissertation on competition of scientific degree of the candidate of food science (doctor of philosophy) in a specialty 181 "Food technology" – Sumy National Agrarian University, Sumy, 2022.

The thesis is devoted to providing development and scientific justification of meat production technology with improved functional and technological properties at the stage of embryonic development (or at the stage of formation of pig offspring). Pork quality is related to human health and is affected by many factors. The distribution and deposition of adipose tissue are the key factors affecting pork quality, and the fat content can affect the meat quality of pigs by changing the juiciness, tenderness, flavor and palatability of pork.

The first part introduces the factors that affect the formation of meat quality from the aspects of fat formation and its regulation, VD_3 physiological function and lipid metabolism, maternal nutrition level affecting the development of adipose tissue and skeletal muscle, animal meat quality characteristics and its influencing factors.

The study was conducted to investigate the effects of maternal VD_3 status during pregnancy on meat quality attributes, technological properties, and processing performance in offspring pigs by feeding trials, slaughter experiment, and meat quality analysis technology. The objective of this study was meat production technology with given functional and technological properties, the level of VD_3 in sows during pregnancy, lipid metabolism and the quality of offspring meat, and to supply scientific basis and implementation plan for early nutritional regulation technology of pork quality characteristics in offspring pigs.

The second part expounds the scientific problems, clarifies the selection of research objects and directions, and puts forward the scientific methods of applied research. According to the research purpose of the experiment, a complete work plan

was formulated, including feeding experiment, slaughtering experiment, analysis technology of potential factors related to meat quality characteristics, physical and chemical indexes of meat quality and relevant methods of storage capacity, in order to determine the best test scheme and implementation conditions. In order to achieve this test task, the research method and technical route were confirmed and described in detail.

The third part presents the results of the effect of VD_3 on the potential food factor of pork. It was found that at the age of 28 days, the concentrations of serum FT3, IGF-I and FT4 and the expression of *PPARγ* in LD group were higher than those in ND group and HD group, while the expression of *ZFP423* and *VDR*, serum 25 (OH) D, leptin and insulin levels in LD group were significantly lower than those in ND group (P<0.05). In addition, the *FAS/HSL* mRNA expression ratio and *FAS* expression of piglets in HD group were higher than those in LD group and ND group (P<0.05).

At 150 days of age, compared with the offspring of ND and HD groups, the LD offspring had higher *FAS/HSL* mRNA expression ratio and *FAS mRNA* expression in subcutaneous adipose tissue, higher *HSL mRNA* expression in the *longissimus dorsal* muscle, and lower *FAS/HSL* mRNA expression ratio in the *longissimus dorsal* muscle. In addition, the average back fat thickness (**ABFT**), carcass fat, serum leptin and insulin levels of LD group were higher than those of ND or HD groups, while the levels of serum triglycerides (**TG**), free fatty acids (**FFA**) and intramuscular fat (**IMF**) were lower. Additionally, IMF content in offspring was negatively correlated with *FAS/HSL* mRNA expression ratio, while carcass fat content and ABFT in offspring were positively correlated with *FAS/HSL* mRNA expression ratio. At the same time, the carcass fat content of offspring pigs was positively correlated with *FAS* mRNA expression, while ABFT was negatively correlated with *FAS* mRNA expression.

Maternal high-dose VD_3 supplementation decreased drip loss, shear force, total saturated fatty acids (**SFA**) content and n-6:n-3 ratio, while increased marbling score, subjective color score, *longissimus* muscle area, total monounsaturated fatty acids (**MUFA**) content, polyunsaturated fatty acids (**PUFA**) content, and the PUFA: SFA ratio.

Maternal high-dose VD_3 can significantly reduce the shear force, drip loss, total n-6: n-3 ratio and total saturated fatty acid (SFA) content of offspring pigs, and significantly increase the subjective color score, marbling score, total polyunsaturated fatty acids (**PUFA**) content, total monounsaturated fatty acids (**MUFA**) content, *longissimus dorsi* muscle area and the total PUFA: SFA ratio.

Through the study of potential food factors affecting pork quality, we found that Maternal VD_3 status during pregnancy have long-lasting impact on meat quality function and technological properties in offspring pigs. High-dose VD_3 maternal feeding can improve the pork quality function and technological properties of offspring by regulating key genes and serum hormones related to adipose tissue deposition. By adjusting the maternal VD_3 level during pregnancy, the pork quality characteristics of offspring were improved, and the scientific feeding scheme of VD_3 in early pregnancy sows was optimized.

The fourth part presents the results of physical-chemical indicators of meat quality and its ability to be stored.

The quality characteristics of *longissimus dorsi* muscle of offspring pigs during post slaughter storage showed that the water holding capacity (WHC) and pH value of HD group were higher than those of LD group, while the relaxation time of T_{21} in HD group was lower (P<0.05); Compared with ND group, the shear force in HD group was lower, while that in LD group was higher (P<0.05), Compared with LD group, the a* values in HD group and ND group were higher, while the b*, L* values and T_{22} relaxation time were lower (P<0.05). At the same time, postmortem storage after slaughter, the pH value in ND group, WHC in LD group, a* and L* value in ND and LD groups were decreased, but the b* value in ND and LD groups, T_{22} relaxation time in LD, ND and HD groups were increased (P<0.05).

The quality characteristics of the *longissimus dorsi* muscle of the offspring during frozen storage showed that the cooking loss, thawing loss, T_{22} and T_{21} relaxation time, b*, L*values of the *longissimus dorsi* muscle of the offspring increased significantly (P<0.05), while the shear force and a * values of the *longissimus dorsi* muscle of the offspring decreased significantly (P<0.05). In addition, the thawing loss,

b* and L* values, T_{22} relaxation time and shear force of *longissimus dorsi* muscle in LD group were higher compared with that in HD group (P<0.05). Meanwhile, compared with LD and ND groups, the offspring of HD group had lower T_{21} relaxation time and cooking loss of *longissimus dorsi* muscle (P<0.05), while LD group had lower a* value compared with HD and ND groups during frozen storage (P<0.05).

The quality properties of pork batters of offspring during cold storage period showed that compared with LD group, the L^*value, cooking loss, T_{22} and T_{21} relaxation time of HD group were lower, while hardness, a^* value, springiness, cohesiveness, and chewiness in pork batters of HD group were higher (P<0.05). Additionally, b^* values and cooking loss of ND and LD groups, T_{22} relaxation time and TPA index of LD, ND group and HD group increased with cold storage, while L* and a* values of ND group and LD group decreased with cold storage (P<0.05).

It is hereby proved that maternal high-dose VD_3 (3200 IU/kg basal diet) supplementation could improve meat quality function and technological properties, and prolong the freezing storage time of pork, and shelf life of pork and pork batters in offspring.

The fifth part presents the recommendations for improving the technology of meat production with given functional and technological properties. The 41st day gestation sow is the key window period for regulating the quality function and technical characteristics of offspring pork. From the 41st day of pregnancy to the farrowing period of sows, adjusting the level of VD_3 supplementation to 3200 IU/kg basic diet can significantly improve the quality characteristics and production performance of offspring pork.

Key words: pigs, pig offspring, pork, meat, fattening, meat quality, vitamin D3, pregnancy, fatty acids, genes, sensory evaluation, meat quality, technological indicators, nutritional value of meat, meat storage.

LIST OF PUBLICATIONS

SCOPUS / Web of Science publications

1. Guo, LP., Miao, Z.G., Ma, H.J., Melnychuk, S. (2020). Effects of maternal vitamin D_3 during pregnancy on *FASN* and *LIPE* mRNA expression in offspring pigs. *The Journal of Agricultural Science*, 158 (1-2), 128-135. DOI: https://doi.org/10.1017/S0021859620000210. (*The applicant participated in research, analysis of the results and writing the article)*.

2. Guo, LP., Miao, Z.G., Ma, H.J., Melnychuk, S. (2021). Effects of maternal vitamin D_3 status on quality traits of longissimus dorsi muscle in offspring pigs during postmortem storage. *Livestock Science*, 243, 104372. DOI: https://doi.org/10.1016/j.livsci.2020.104372. (*The applicant participated in research, analysis of the results and writing the article*).

3. Guo, LP., Miao, Z.G., Ma, H.J., Melnychuk, S. (2020). Effects of maternal vitamin D-3 concentration during pregnancy on adipogenic genes expression and serum biochemical index in offspring piglets. *Journal of Animal and Feed Sciences*, 29, 125-131. DOI: https://doi.org/10.22358/jafs/124041/2020. (*The applicant participated in research, analysis of the results and writing the article*).

4. Guo, LP., Miao, Z.G., Ma, H.J., Melnychuk, S. (2020). Effects of maternal vitamin D-3 on quality and water distribution in pork of offspring pigs during frozen storage. *Journal of Animal and Feed Sciences*, 29 (4), 330-337. DOI: https://doi.org/10.22358/jafs/130781/2020. (*The applicant participated in research, analysis of the results and writing the article*).

5. Guo, LP., Miao, Z.G., Ma, H.J., Melnychuk, S. (2021). Effects of maternal vitamin D_3 status on meat quality and fatty acids composition in offspring pigs. *Journal of Animal and Feed Sciences*, 30, 173-178. DOI: https://doi.org/10.22358/jafs/138652/2021. (*The applicant participated in research, analysis of the results and writing the article*).

Conference papers

6. Liping Guo，Sergiy Melnychuk，Hanjun Ma. (2020). Effects of maternal vitamin D3 during pregnancy on meat quality attributes and chemical composition of longissimus dorsi muscle in offspring pigs. Asia Pacific meat science and Technology Conference and the 18th China Meat Science and Technology Conference (Hefei, China, 2020). (*PhD participant in carrying out of experimental research, processing of results, and writing the article*).

7. Liping Guo，Sergiy Melnychuk，Hanjun Ma (2020). Effects of Maternal Vitamin D3 during Pregnancy on Carcass Characteristics，Meat Quality and Fatty Acids Composition in Offspring Pigs. Asia Pacific meat science and Technology Conference and the 18th China Meat Science and Technology Conference (Hefei, China, 2020). (*PhD participant in carrying out of experimental research, processing of results, and writing the article*).

8. Liping Guo，Sergiy Melnychuk，Hanjun Ma (2020). Effects of maternal vitamin D3 status on quality traits and water distribution of longissimus dorsi muscle in offspring pigs during postmortem storage. 2020 Asia Pacific meat science and Technology Conference and the 18th China Meat Science and Technology Conference (Hefei, China, 2020). (*PhD participant in carrying out of experimental research, processing of results, and writing the article*).

THE LIST OF SYMBOLS

MSCs	Mesodermal stem cells
PPAR	Peroxisome proliferator-activated receptor
C/EBP	CCAAT enhancer-binding protein
FAPs	Fibro/adipogeneic progenitors
IMF	Intramuscular fat
ZFP423	Zinc finger protein 423
SREBP-1	Sterol regulatory element binding protein 1
PPRE	PPARγ response element
RXR	Retinol X-receptor
FABP4	Fatty acid binding protein 4
AP2	Adipocyte lipid-binding protein 2
BMPs	Bone morphogenetic proteins
TGFβ	Transforming growth factor β
HSL	Hormone sensitive lipase
FAS	Fatty acids synthase
FFAs	Free fatty acids
ADD1	Adipocyte determination and differentiation 1
CPT1	Carnitine palmitoyltransferase-1
KLFs	Kruppel like factors
AMPK	AMP-activated protein kinase
Pref-1	Preadipocyte factor-1

ERK	Extracellular signal-regulated kinase
LPL	Lipoprotein lipase
IGF-I	Insulin-like growth factor I
FT3	Free triiodothyronine
FT4	Free thyroxine
GADD45α	Growth arrest and DNA damage inducible protein 45 alpha
VDR	Vitamin D receptor
ETO	Eight twenty-one
SCD1	Stearoyl-CoA desaturase-1
ASC	Adipose derived stem cells
RARs	Retinoic acid receptors
RAREs	Retinoic acid response element
ING1	Inhibitor of growth protein 1
PDGFRα^{+}	Platelet-derived growth factor α positive
DKK1	Dikkopf 1
SFRP2	Secreted frizzled-related protein 2
WHC	Water holding capacity
LF-NMR	Low field nuclear magnetic resonance
L*	Lightness
a*	Redness
b*	Yellowness
TG	Triglycerol
ABFT	Average back fat thickness

SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
TPA	Texture profile analysis
NRC	National research council
DE	Digestible energy
CP	Crude protein
Lys	Lysine
Met	Methionine
Cys	Cystine
ACTB	Beta-actin
SEM	Standard error of the mean.
ACC	Acetyl-COA carboxylase
GPDH	Glycerol-3-phosphate dehydrogenase
MyoD	Myogenic Differentiation
WAT	White adipose tissue
BAT	Brown adipose tissue
MEFs	Mouse embryonic fibroblasts
PGC-1	PPARγ coactivator-1
RIP140	Receptor-interacting protein 140

CONTENTS

INTRODUCTION

Relevance of the topic. Pork quality is closely related to human health. The distribution and deposition of adipose tissue are the key factors affecting pork quality. Excessive subcutaneous fat deposition will reduce the eating quality of pork and damage the health of consumers. However, higher intramuscular fat content can improve the tenderness, marbling, juiciness and flavor of pork, which is beneficial to human health.

The content of fat in pig muscle mainly depends on the number of precursor adipocytes and the ability of fat synthesis and accumulation. Therefore, how to effectively improve the number of pig intramuscular precursor adipocytes, fat synthesis and accumulation capacity, increase intramuscular fat content, and produce high-quality pork products through nutritional regulation is a serious challenge for livestock producers.

Developmental biology research shows that pig skeletal muscle and adipose tissue originate from the directional differentiation of embryonic mesodermal mesenchymal stem cells (MSCs). The proportion of MSCs differentiated into adipocytes plays a very important role in pig intramuscular adipogenesis. A large number of MSCs differentiated into myogenic progenitor cells at the early embryonic stage of pigs, entered the skeletal muscle development process, and were basically fixed at 90 days of age; Adipose tissue developed late. At 45/60 days of age, only a few MSCs differentiated into fibroblasts/fat progenitors, entered the adipogenesis process, differentiated into fat deposition sites, and formed intramuscular fat in the late growth and development of pigs. In view of the common time coincidence point of differentiation and development of porcine fetal MSCs into skeletal muscle and fat, namely 45/60-90 days of gestation, this period of time has created an excellent time "window" for us to further study the nutritional regulation mechanism of adipogenic differentiation of MSCs.

Intramuscular fat deposition of pig is affected by many factors, including genetic background, nutrition, external environment and feeding methods. More and more evidence showed that VD3 is a potential nutritional regulator affecting fat production,

and it plays an important role in the regulation of animal adipocyte anabolism. The key is that VD3 is beneficial to form the function and technical characteristics of pork at the formation stage of pig offspring. Therefore, vitamin D3 in pork will be better absorbed in this window period. Therefore, the purpose of this experiment is to reveal the formation of functional and technical properties of pork by the influence of Vitamin D3 on the differential maternal level of pigs by studying the influence of maternal VD3 levels on the quality characteristics of offspring pork during pregnancy. So as to provide a scientific implementation plan for improving the physical and chemical properties, storage and processing performance of the offspring pork.

Connection of work with scientific programs, plans, topics. Work carried out in according with the main directions of scientific research of National Natural Science Foundation of China, Sumy National Agrarian University and Henan Institute of Science and Technology within the framework of scientific topics: self-reported topic 《Vitamin D3 and its molecular regulation mechanism in porcine fetal skeletal muscle derived MSCs adipogenesis (U1604102)》and 《Molecular regulatory mechanisms during porcine intramuscular fat deposition (31572417)》. To study the effects of maternal VD3 status during pregnancy on meat quality function and technological properties in offspring pigs, so as to provide a scientific basis for improving the nutritional value of pork.

The purpose and objectives of the study. The purpose of the dissertation work is that development and scientific justification of meat production technology with improved functional and technological properties at the stage of embryonic development (or at the stage of formation of pig offspring).

In order to achieve the main research goal, it was necessary to solve a number of interrelated tasks:

- Analyze functional and technological indicators of meat quality;
- Study the impact of VD_3 on the ability of meat to be stored;
- To improve the technology of meat production with given functional and technological properties;

Object research – Meat production technology with given functional and technological properties, the level of VD3 in sows during pregnancy, lipid metabolism and the quality of offspring meat.

Subject of research – Pork meat, functional and technological properties of meat, differential maternal status of VD3 sow, offspring

Research methods. The RT-PCR, radioimmunoassay and enzyme-linked immunosorbent assay were used to clarify the impacts of VD_3 levels of pregnant sows on factors related to the formation of meat quality function in offspring. LF-NMR, pH meter, CLM-4 digital explicit muscle tenderness meter, colorimeter was used to clarify the influences of VD_3 status of pregnant sows on meat quality properties of offspring during cold and frozen storage period. Gas chromatograph was used to clarify the effects of maternal VD_3 status on the composition of fatty acids in *longissimus dorsi* muscle of offspring, and experiment planning methods and mathematical processing of experimental data computer programs.

Scientific novelty of the obtained results. This thesis established the rules of maternal VD_3 status during pregnancy on adipogenic genes expression, serum biochemical index, carcass characteristics, and meat quality in offspring for the first time. Confirmed the best addition level of VD_3 in pregnant sows affecting the quality of offspring pork, whose pork quality index are formed by changing maternal VD_3 levels.

Practical significance of the results. Based on the results of theoretical and experimental studies provide a scientific basis for the early nutritional regulation of pork quality and process-ability (by regulating the level of VD_3 in pregnant sows), and maternal high-dose VD_3 (32000 IU/kg basal diet) during pregnancy (41st day of pregnancy) could improve meat quality and processing performance in offspring pigs by altering lipid metabolism. Up to day, the technology of nutritional regulation of VD_3 in pregnant sows to improve the quality of offspring pork has been widely applied in Ji'an Nonghao Agricultural Development Co., Ltd, Henan heiyuan agriculture and animal husbandry technology Co., Ltd, and Chunfa farm in Shenqiu County. The

application of the technology in these enterprises has significantly improved growth performance, meat quality of pigs, and the economic benefits of enterprises.

The applicant's personal contribution is in planning an experiment, organization and conduct of analytical and experimental research in laboratory and production conditions, analysis, processing and generalization results, formulating conclusions and recommendations, preparing materials for publication, introduction of new technologies into production.

Publication. According to the dissertation work 8 scientific publications, 5 of them in periodical scientific publications indexed in Web of Science or Scopus and 3 abstracts of reports at scientific and practical international conferences.

Structure and scope of the dissertation. The dissertation contains an annotation, introduction, 3 sections, conclusions and a list of sources used, which including 276 foreign ones. Main content dissertation is laid out on 112 pages of printed text, contains 23 tables, 15 figures.

SECTION 1. ANALYSIS OF FACTORS AFFECTING THE FORMATION OF MEAT QUALITY

Pork quality is related to human health and is affected by many factors. The distribution and deposition of adipose tissue are the key factors affecting pork quality, and the fat content can affect the meat quality of pigs by changing the juiciness, tenderness, flavor and palatability of pork.

1.1 Adipose tissue formation and its regulatory mechanism

Adipose tissue is the main form of energy storage in animals. When the energy materials englobed by animals exceed their required consumption, they can be stored in the form of fat. While the intake of energy materials cannot meet the needs of physiological activities, the fat stored in the body is used for oxidation to meet the energy needs of animals. Fat accumulation is determined by the dynamic balance between degradation and synthesis [1]. Once the original balance is broken, it will lead to the increase or decrease of fat deposition, which will affect the meat quality of animals. Pork is an important source of food for people, its quality is directly related to human health. The amount of fat deposition in pigs is the main factor affecting pork quality. Therefore, understanding adipose tissue formation and its regulatory mechanism in pig and improving pork quality can provide a scientific theoretical basis for producing high-quality pork to meet people's living needs.

1.1.1 Classification and composition of adipose tissue

Adipose tissue is a complete functional body composed of adipocyte, connective tissue matrix, nerve tissue, vascular stromal cells, and immune cells. Emerging evidence showed that the adipose tissue is an important the storage organ of energy in animals, it is also a crucial endocrine organ, which can secrete adipokines to regulate the energy balance of the body. According to the different fat deposition sites in livestock, it can be divided into four main parts: subcutaneous, visceral, intramuscular, and intermuscular adipose tissue. Visceral fat is more widely distributed than subcutaneous fat and contains more immune and inflammatory cells. Subcutaneous fat

is the main energy storage organ and important endocrine organ of the body. Lipid can be deposited in intermuscular and intramuscular adipocytes, and the intramuscular adipocytes can store triacylglycerol [2]. The hormones and cytokines secreted by subcutaneous fat play a crucial function in regulating body's temperature, energy balance, insulin sensitivity and so on [3]. In addition, according to different biology functions, adipose tissue mainly contained white, brown, and beige adipose tissue. White fat and brown fat are derived from MSCs. The adipose tissue distributed in abdominal cavity and thoracic cavity is called visceral fat, and they mainly include omental fat, perirenal fat and mesenteric fat. White adipose tissue is widely accumulated in animals, which is the main form of fat in adult animals. The increase in the number or volume of white adipocytes in animals will cause excessive aggregation of white adipose tissue, leading to obesity, which will cause serious damage to the health of animals.

Brown adipose tissue is mainly distributed between scapulae, neck and around aorta. It will not cause other tissue dysfunction while consuming energy, and plays an antagonistic role in obesity. When brown adipose tissue was excised, mice could cause overeating and obesity. In addition, beige fat cells contain brown fat droplets, whose function is between white and brown adipose tissue. They can not only store energy, but also consume energy.

Adipose tissue mainly exists in the form of triglycerides in animals and is the most important energy source for normal growth of animals. For meat livestock and poultry, the content and composition difference of fat not only determines the quality grade and nutritional value of meat products, but also related to the economic benefits of livestock and poultry. The main component of subcutaneous fat is neutral fat. In addition, there are a small amount of cholesterol, diglyceride, monoglyceride and free fatty acid. The composition of intermuscular fat is like that of subcutaneous fat. Intramuscular fat is composed of intramuscular adipose tissue and fat in muscle fibers. The adipocytes of intramuscular adipose tissue are arranged along the muscle fibers and are basically composed of triglycerides, while the fat in the muscle fibers is

composed of triglyceride droplets in the muscle plasma, membrane lipids (mainly phospholipids) and cholesterol.

1.1.2 Adipogenesis

Adipose tissue is usually referred to as fat, the main storage site of triglyceride, is an important metabolic organ, which also plays a crucial role in maintaining glucose homeostasis and energy dynamic balance. Therefore, adipose tissue is the main storage site of triacylglycerols. Meanwhile, it is can be used as an endocrine organ to release fatty acids and proteins [4]. Adipose tissue is composed of a variety of cells, mainly including preadipocytes, mature adipocytes, macrophages, fibroblasts, immune cells, and so on. The main cells types in adipose tissue are mature adipocytes.

Adipogenesis is a highly ordered process, in which adipogenic differentiation is a key link. Adipogenic precursor cells (pluripotent stem cells and preadipocytes) must undergo adipogenic differentiation to develop into adipocytes. The essence of adipocyte differentiation is the change of gene expression in cells. Under the condition of adipogenic induction, many regulatory factors (signal pathways and transcription factors) are activated. These regulatory factors act on the downstream target genes and change the expression of genes related to adipose differentiation. Previous studies have found that adipogenesis is a complex process, which is affected by the regulatory network composed of a variety of transcriptional regulatory factors. At present, most studies focus on the stage of cell differentiation, that is, the stage of transformation from preadipocytes to mature adipocytes. In adipose tissue, the differentiation of preadipocytes into mature adipocytes, the PPAR (peroxisome proliferator-activated receptor), Kruppel-like factor proteins, and C/EBP (CCAAT enhancer-binding protein) play important regulatory roles [4-6]. Among them, PPAR is the core transcription factor, which plays the role of molecular "switch" and is necessary for adipogenic differentiation.

1.1.3 Structure and function of adipose tissue

White adipose tissue includes subcutaneous, visceral, and intramuscular adipose depots. Intramuscular adipose tissue, often called marbling, is mainly accumulated in adipocytes in skeletal muscle, which affects the flavor, tenderness, juiciness of pork [7].

Marbling is the important factor affecting the meat quality of animal. Subcutaneous fat is distributed under the skin [8]. Meanwhile, visceral fat is distributed around visceral organs [9]. The shape of white adipocytes is spherical cells, and its size mainly relies on the size of individual lipid droplets stored in them (white adipocytes). And the lipid droplets are composed of triglycerides, accounting for more than 90% of the cell volume. Subcutaneous fat, internal fat, and intramuscular fat have important economical and physiological roles in meat animal production.

Brown adipose tissue can help newborns and small mammals resist cold temperatures, which is mainly used for thermogenesis and thermoregulation of newborn [10], and it is considered to be absent or at least of no relevance in adults. However, recent study have demonstrated that brown adipose tissue plays an significant function in energy homeostasis of adults, which indicated that adults also have metabolically active brown adipose tissue[11]. So, brown adipose tissue is considered as a crucial target for the treatment of obesity and fat metabolic disorders. Brown adipocytes belong to multiple small vacuoles contain triglycerides, and their cells are scattered in many small lipid droplets. In addition, Brown adipose tissue includes more capillaries than white adipose tissue, because it requires more oxygen demand. Brown fat seems to play a key role in the warming mechanism of blood supply to important organs[12].

1.1.4 From MSCs to mature adipocytes

MSCs have totipotency and multidirectional differentiation potential, which have the ability to develop into many cell types (including myocytes, adipocytes, osteocytes, and chondrocytes). They can form adipose and muscle tissue through adipogenic/myogenic directional differentiation. Growing evidence shows that aidpogenic commitment and differentiation in embryo and fetus is one of the important factors influencing the formation of intramuscular fat deposition phenotype.

Previous reports have demonstrated that adipocytes derived from multipotent MSCs, and there are two stages (the determination and the terminal differentiation) for determination of MSCs into mature adipocytes [13]. The first stage is the commitment of MSCs into preadipocytes, which is called the determination period. The second stage

is the terminal differentiation, in which the preadipocytes differentiate into mature adipocytes [14]. Preadipocytes are cells that do not have lipid droplets. Whereas, mature adipocytes are unicellular adipocytes, which filled with a large lipid droplet. The stages of adipogenesis from MSCs to mature adipocytes was shown in Figure 1-1.

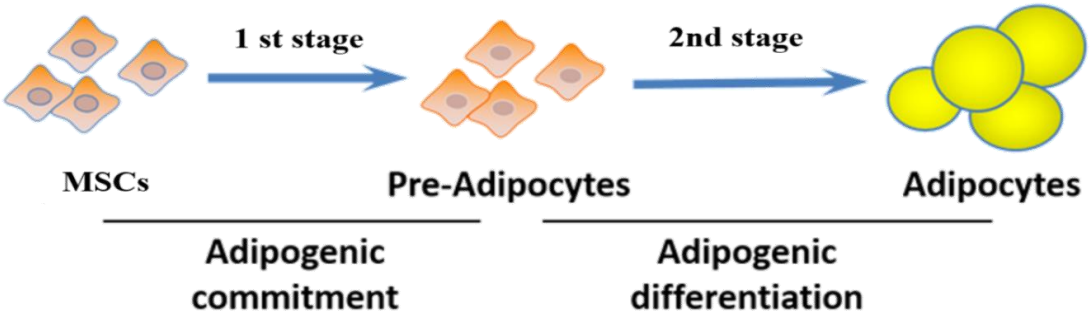


Figure 1-1 The stages of adipogenesis from MSCs to mature adipocytes

1.1.5 Types of adipocytes

Although adipose tissue includes a variety of cell types, adipocytes (the main storage site of triacylglycerols) are dominant. Mammalian adipocytes can be divided into three types: white, brown, and beige adipocytes respectively. Of them, white adipocytes can convert fat into energy in the metabolic process to meet the needs of animal muscle tissue and other body functions. Therefore, when the energy contained in the food is too much, these white adipocytes will grow exponentially. At the same time, a single cell will become larger, which will eventually lead to obesity. Most mammals contain very little white adipose tissue at birth (less than 5% of birth weight).

Brown adipocytes are a kind of adipocytes that produce heat. The cells contain a great deal of mitochondria, which can oxidize long-chain fatty acids converted from triglycerides. Most mammals are born with brown adipose tissue, but newborn pigs do not contain brown adipose tissue. Brown adipocytes develop in brown adipose tissue in response to cold stimulation [15].

The comprehensive effects of adipocyte proliferation and hypertrophy lead to the growth and development of adipose tissue. The increase in the number of adipocytes is mainly realized by the differentiation and proliferation of preadipocytes. The increase of lipids in adipocytes (mainly triglycerides) can increase the volume and weight of cells.

Recent study found that beige adipocytes in white adipose tissue can be induced to develop under the stimulation of cold environmental conditions. So, beige adipocyte is also defined as a cluster of adipocytes expressing uncoupling protein 1 (UCP1) located outside the traditional brown adipose tissue depots. Beige adipocytes also have the ability to change energy into heat [16]. The activity of brown and beige adipocytes decreases metabolic diseases in mice, containing obesity, so it is considered to related to weight loss in humans. Exposure to cold conditions is a powerful trigger of beige and brown fat thermogenesis, and some mice lacking UCP1 cannot survive under cold conditions.

1.1.6 Adipocyte development

Growing evidence has demonstrated that MSCs have the potential to differentiate into different cell lineage, such as adipocyte lineage, myoblasts lineage and osteoblasts lineage [17-19]. These finding indicated that muscle cell and adipocyte are originated from the same MSCs. In the early developmental period of skeletal muscle (fetal and neonatal stage), most of the MSCs differentiate into myogenic cells, but only a small part of them differentiates into adipocytes. Intramuscular adipocytes were mainly generated at the fetal and neonatal stages [20], they would provide the sites for intramuscular fat (IMF) accumulation that generate marbling at the fattening stage in offspring [21]. IMF content is associated with the size and number of intramuscular adipocytes, and fetal programming plays a pivotal role in the number of adipocytes of offspring. Therefore, fetal developmental period has an important impact on the number of adipocytes of offspring. The ratio of MSCs commitment to adipogenic lineage determines IMF content, and increasing adipogenesis in fetal muscle tissue could enhance the number of intramuscular adipocytes and increase meat quality [7, 22].

Other studies has also found that fibro/adipogenic progenitors (FAPs) are derived from endothelial mesenchymal progenitors in lateral mesoderm, and can generate fibroblasts, resident fibro/adipogenic progenitor cells, and adipocytes[14, 23]. The majority of FAPs are generated during fetal development [24]. Commitment of MSCs into fibro/adipogenic cell lineages was shown in Figure 1-2.

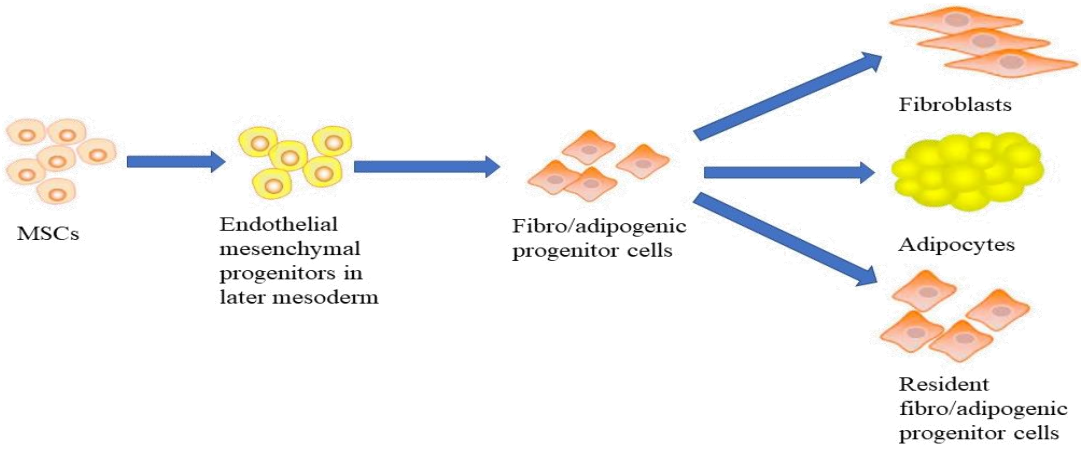


Figure 1-2 Commitment of MSCs into fibro/adipogenic cell lineages

In addition, some stem cells also exist in the bone marrow and vascular stroma of adipose tissue. When properly stimulated, they will undergo a multi-step commitment process, in which the stem cells are limited to the adipocyte lineage. Adipocyte lineage generates preadipocytes, which differentiate into adipocytes after undergoing multiple rounds of mitosis. The transformation of pluripotent stem cells into adipocyte lineage is usually regulated by many factors of differentiation or inhibitors. The commitment process of adipocytes can be regulated by many factors, such as BMP4, BMP2 (bone morphogenetic protein) and downstream signaling pathways [25]. Whereas, hedgehog signaling has inhibition, and Wnt seems to activate in commitment and inhibit adipocyte differentiation.

1.1.7 Molecular regulation of adipogenesis

Adipogenesis is adjusted by genetic, gene, translation factor, molecular signaling pathway, nutrition and environmental factors [26]. Adipocyte differentiation is accompanied by changes in cell function and structure, during which many proteins are expressed [27]. Many changes occur through a variety of molecular events at the gene expression level. Meanwhile, many studies have confirmed that adipocyte differentiation is a highly delicate regulatory process [28-30]. A variety of transcription factors, signaling pathways and microRNAs also play crucial functions in the process of adipocyte differentiation. For example, Zinc finger protein 423 (ZFP423), and peroxisome proliferator-activated receptor γ (PPARγ) play decisive roles in initiating the transformation of precursor adipocytes into mature adipocytes.

1.1.7.1 ZFP423

ZFPs participate in different cellular reactions, such as cell growth, proliferation, differentiation, immunity, metabolism, and adipogenesis. ZFP423 is a crucial transcription factor for committing MSCs to preadipocyte [31], and it can play a role as a key transcriptional regulator involved in adipogenesis. ZFP423 was recently found to be directly involved in early adipose determination. The fetal stage of animals is the key period for committing MSCs to preadipocytes. During the commitment stage of adipocyte, ZFP423 plays an important regulatory function [3]. Previous reports have demonstrated that ZFP423 could increase preadipocyte determination by activating the expression of PPARγ in undifferentiated cells [31]. So, ZFP423 can promotes the PPARγ expression and adipogenic markers. Recently, it is found that ZFP423 regulates the expression of PPARγ by amplifying BMP signal pathway, play a key role in modulating the adipogenic potential determination of precursor cells. The expression of ZFP423 in preadipocytes increased significantly, but its expression did not change during the transformation from preadipocytes to adipocytes, indicating that ZFP423 is an important transcriptional regulator for preadipocyte commitment [32]. ZFP423 contains a binding domain of SMAD, which is necessary for bone morphogenetic protein 4 (BMP4)-dependent lipogenesis. However, ZFP423 mutant lacking SAMD binding domain can still induce PPARγ in NIH 3T3 cell line and enhance adipogenesis. These results suggested that ZFP423 may improve adipogenesis by BMP dependent and BMP independent manner. However, the upstream signal of ZFP423 gene expression and its molecular regulation mechanism are still unclear.

1.1.7.2 PPARγ

PPARγ is an important ligand-dependent transcription factor and belongs to one of the members of the nuclear receptor super-family. In addition, it is a target of thiazolidinedione compounds (TZDs), which are for anti-diabetes drugs. Many studies have confirmed that the expression of PPARγ is essential for the formation of white adipocyte tissue and brown adipocyte tissue [33-35]. Recent study found that PPARγ has

important regulatory functions during the adipocyte differentiation [36]. Although PPARγ play an important regulatory role in the early period of adipocyte differentiation, but it is not necessary in mature adipocytes. So, PPARγ probably is a precise regulator of adipogenesis[37]. In addition, previous reports found that embryos lacking PPARγ could not form adipose tissue in chimeric mice [38]. Other study found that PPARγ knockout in mouse embryonic fibroblasts (MEFs) resulted in the loss of adipocyte differentiation [39]. Up to now, no factors causing fat formation due to lack of PPARγ has been found. The PPARγ can play a transcriptional regulatory role by specifically targeting the PPRE (PPARγ response element) in the promoter region of its target gene [40]. In adipocytes, PPARγ transcripts regulate the expression of glycometabolism-related genes and promote triglyceride accumulation by forming a heterodimer with RXR (retinol X-receptor) or by binding to its co-activator PGC-1 (PPARγ coactivator-1) or co-inhibitor RIP140 (Receptor-interacting protein 140) [41]. However, the endogenous ligands of PPARγ in adipocytes have not yet been found. Although some metabolic intermediates such as polyunsaturated fatty acids, arachidonic acids, and prostaglandins, are considered ligands, most of these molecules have a low affinity for PPARγ. Their expression in adipocytes is also low, and their physiological functions remain unclear. Meanwhile, PPARγ is considered as a key regulatory factor of preadipocyte differentiation into mature adipocytes, and plays a key role in maintaining the phenotype of mature adipocytes[31].

1.1.7.3 Fatty acid binding protein 4 (FABP4)

FABP4 is a cytoplasmic fatty acid chaperone expressed in adipocytes and myeloid cells. It belongs to a subtype member of the fatty acid-binding protein family. Previous study shown that FABP4 deficient mouse preadipocytes increased the expression of PPARγ, and it significantly enhanced adipogenesis. These results have demonstrated that FABP4 regulates adipogenesis by down-regulating PPARγ [42]. Previous study reported that lipid transporter FABP4 is a major target gene of PPARγ, and FABP4 is almost only induced by PPARγ in adipocytes and macrophages [43]. As a fatty acid chaperone, FABP4 is related to the effects of intracellular lipids on biological

targets and signal pathways. FABP4 decreases PPARγ expression and adipogenesis, which is negatively correlated with PPARγ of adipose tissue. Recently, studies have shown that FABP4 is a secretory hormone which plays a role in maintaining the homeostasis of glucose. It is a crucial node to promote communication between distant organs and energy storage system to deal with life-threatening situations[44].

Additionally, FABP4 is abundant in adipocytes. It keeps adipocyte dynamic balance and regulates lipolysis and lipogenesis by interacting with hormone sensitive lipase (HSL) and PPARγ, respectively [45]. Under the conditions of lipolysis, it is suggested that FABP4 combines with free fatty acids (FFAs) in the cytoplasm to regulate the inhibitory activity of released lipids on lipolytic enzymes [46]. The mechanism of adipose tissue secretion of FABP4 is closely related to the signal downstream of lipolysis stimulation, which is continuously involved in insulin resistance, stress and obesity[44].

FABP4 can cause renal interstitial fibrosis by regulating inflammation and lipid metabolism[47]. There is a positive correlation between FABP4 and FFAs. FABP4 in high dosage can directly impair endothelial cells, and damaged endothelial cells can further improve the increase of FABP4 level, and then cause the deposition of triglycerides and cholesterol, accompanied by lipid metabolism disorder[48]. PPARγ can be negative feedback regulated by FABP4, and it can also modulate the transport, oxidation and decomposition of fatty acids by regulating fatty acid transporters, fatty acid binding proteins and CPT1 (carnitine palmitoyltransferase-1) expression [49]. Therefore, FABP4 plays an important role in regulating inflammation and lipid metabolism.

1.1.7.4 Fatty acids synthase (FAS) and hormone-sensitive lipase (HSL)

Intramuscular adipocytes were mainly generated at the fetal and neonatal stages [20], they would provide the sites for IMF accumulation that generate marbling at fattening stage in offspring [50]. Previous study reported that adipose tissue occurred before mid-gestation in many mammals, and maternal malnutrition or over-nutrition affected overall fat accumulation of offspring. These results suggested that lipid synthesis and degradation in offspring were impacted by maternal nutrition. Previous

reports demonstrated that adipose tissue deposition depends on the balance between lipid synthesis and degradation [51, 52]. This process is mainly regulated by *FAS* and *HSL*. The *FAS* exerts a vital role in *de novo* lipogenesis of mammals [53]. Whereas, the *HSL* plays an important role in hydrolyzing TG to FFA in adipose tissue, and regulates the lipolysis of animals [54]. The ratio of *FAS/HSL* mRNA expression and *FAS* mRNA expression was positively related to carcass fat content in pigs [51].

1.1.7.5 Leptin

Leptin, a multifunctional protein, is released by adipocytes, which can regulate energy balance and blood glucose balance. It is mainly secreted by white adipose tissue. Recent studies have proved that leptin can act on the central nervous system, stimulate the hypothalamus through its receptor, control the signal of energy storage, regulate energy metabolism, and reduce body fat deposition. Meanwhile, the lack of leptin in human and mice directly caused serious obesity. In the study of obese (ob) gene mutant C57BL/6J mice, it was found that the level of leptin in plasma was positively correlated with the weight of mice, and the leptin regulates fat accumulation and body weight of animals by affecting metabolism and appetite [55].

1.1.7.6 Other factors

Fat deposition represents a dynamic balance between fay synthesis and fat degradation, which is adjusted by hormonal and non-hormonal factors [1]. Growth factors and hormone play an important role in regulating lipid metabolism and adipogeneses process of animals [56]. Previous report found that leptin is a key regulator of energy homeostasis of mammals, which derives from adipocytes [57]. There is a correlation between circulating serum leptin levels and fat deposition [58]. Serum leptin and insulin concentrations enhanced lipoprotein lipase (LPL) activity and the incorporation of glucose into fatty acids within adipose tissue, while IGF-I (insulin-like growth factor I), FT3 (free triiodothyronine), and FT4 (free thyroxine) can increase energy expenditure altering lipid metabolism pathway, and inhibit fat synthesis by decreasing the activity of lipogenic enzyme in pigs [56].

Moreover, maternal nutrition during pregnancy (intrauterine environment) is a key determinant of fetal growth and maternal nutrient levels can affect the adipose function of offspring. Fat accumulation in body was increased rapidly during neonatal life, and maternal micronutrient deficiency (vitamin D status) was associated with adiposity [59, 60]. Growing evidence has demonstrated that maternal vitamin D levels can alter adipocyte determination and adipose accumulation of offspring [22, 61, 62].

1.2 Physiological functions of vitamin D and adipogenesis

Obesity and vitamin D (**VD**) deficiency are very common all over the world. VD deficiency may lead to obesity and endanger people's physical and mental health. From the cellular level, obesity is caused by fat cell hypertrophy and proliferation, and one of the main reasons for these phenomena is the excessive accumulation of fat in cells. These results suggested that VD may regulate the synthesis and catabolism of adipocytes.

1.2.1 VD Source and synthesis

VD not only maintains calcium homeostasis, regulates cell proliferation and differentiation, but also has endocrine and paracrine functions. VD can be obtained not only from the dietary sources, but also from the endogenous synthesis process in the skin. Previous research found that VD is considered as an essential nutrient, which is necessary for the physiologic function of various organs. It mainly contains two forms which VD_3 and VD_2. The VD_3 is generated from 7-dehydrocholesterol in the skin of animals upon ultraviolet irradiation (280-310 nm light), which presents in the cell membranes of epidermis [63]. However, the VD_2 is mainly generated by plants and fungi [64]. Meanwhile, VD_3 and vitamin D_2 can also be provided by the diet of animals, and fish liver oils, egg yolk and chicken liver are rich in VD.

Animals can only obtain a few fat soluble VD through diet [65]. The main source of animal VD is generated within the skin via the chemical reaction of sunlight-dependence [66]. When the skin is exposed to ultraviolet-B radiation, 7-dehydrocholesterol is converted to VD_3 precursor, which can be further isomerized into

VD_3. It is switched into calcifediol [25-(OH)D] in animals' liver, and then hydroxylated into 1, 25(OH)2D within animal's kidney. Generally, the concentration of 25-(OH)D in serum represents the VD status in individual serum, whereas the bioactive form of VD is 1, 25(OH)2D [67, 68].

1.2.2 VD absorption and metabolism

The small intestine is the main absorption site of VD. Both VD_3 or VD_2 absorbed by intestinal mucosa and VD_3 synthesized in skin enter the blood. The liver of aquatic animals can store a lot of VD, while the liver, kidney, lung, skin, and fat of terrestrial animals can also store VD. The first step in VD metabolic activation is the hydroxylation of carbon 25, mainly in the liver. In addition, VD can have physiological function through hydroxylation of liver and kidney. In the microsomes and mitochondria of hepatocytes, VD_3 generates 25-OH-D3 by the action of 25 hydroxylase; in the mitochondria of renal tubular cells, it is further transformed into 1,25-$(OH)_2$-D_3 through the action of 1-α-hydroxylase, that is a real active form of VD, and its action is similar to steroids [69].

VD not only plays an important regulatory role in intestinal phosphorus and calcium absorption and the regulation of bone mass, but also participates in immune function, cell growth, neuromuscular, and inflammation regulation.[70, 71]. VD could be hydroxylated by enzymes CYP2J2, CYP27A1, CYP27B1 and CYP3A4 of the liver and CYP27B1 of the kidney [72]. In visceral and subcutaneous adipose tissue, these enzymes can be expressed continuously [73, 74]. Previous research found that the CYP27B1 gene is regulated by calcium, hormone, calcitonin, and phosphorus, which is expressed in 3T3-L1 preadipocytes and subcutaneous adipose tissue of lean people [75]. Therefore, the production of active VD may be related to the location of these enzymes in adipose tissue. VD can be converted into 25-hydroxyvitamin D (25(OH)D) in the liver through the hydroxylation of 25 hydroxylase (CY271A1 and CYP2R1). 1a-hydroxlases converted and activated 25(OH)D to 1,25 dihydroxy vitamin D (1,25$(OH)_2$D, and the metabolism of VD as shown in Figure 1-3 [76].

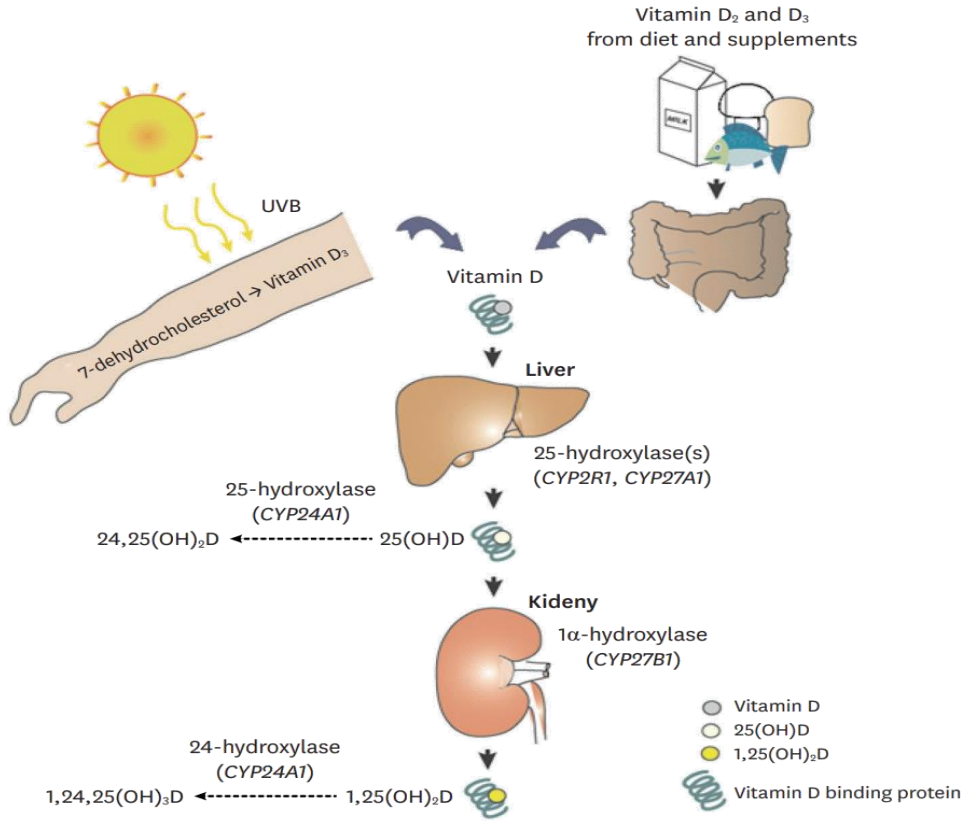


Figure 1-3 Metabolism of vitamin D[76]

Meanwhile, the main rate limiting step of the synthesis of $1,25(OH)_2D$ is 1α-hydroxylation process, and it is strictly regulated by 1α-hydroxylase. The gene encoding 1α-hydroxylase is called CYP27B1, and its deficient mice developed rickets.

Studies have shown that lower serum VD concentrations (blood VD concentrations below 50 nmol/L) are associated with a higher frequency of obesity and overweight[77, 78]. The concentration of 25 (OH) D in blood circulation mainly depends on the content of VD in adipose tissue, which suggested that adipose tissue may affect obesity by regulating VD [79]. 25 (OH) D level is positively correlated with VD intake. Therefore, plasma 25 (OH) D concentrations are usually used as indicators of VD status[80].

1.2.3 VD signalization

As an endocrine system, VD plays a key role in inducing cell differentiation, inhibiting cell growth, immune regulation, lipid metabolism and controlling other hormone systems. The bioactive metabolite of VD is 1, $25(OH)_2D_3$, which can be used as a high affinity ligand for steroid hormones and VD receptor (VDR). VDR is a nuclear biological macromolecule that mediates the biological function of 1,

$25(OH)_2D_3$. Many biological functions of 1, $25(OH)_2D_3$ are realized through VDR mediated regulation of target gene transcription. 1, $25(OH)_2D_3$ can form a heterodimer (VDR-RXR) with RXR after activating VDR. VDR-RXR can bind to the vitamin D receptor response element (VDRE) of a specific gene, thus promoting or inhibiting gene expression. Increasing evidences showed that more than 1000 genes are directly or indirectly regulated by $1,25(OH)_2D_3$, in a variety of physiological process such as cell differentiation, proliferation, apoptosis, immunomodulation, immune response, anti-inflammation, adipogenesis and angiogenesis [21, 22, 81].

1.2.4 VDR

The biological activity of 1,25 (OH) $_2D_3$ requires the participation of VDR (a high affinity receptor). So, the biological function of 1, $25(OH)_2D$ is regulated via binding to VDR [82]. The VDR belongs to an ancient member of the steroid hormone nuclear receptor superfamily, which can play biological function as a ligand activated transcription factor [83].

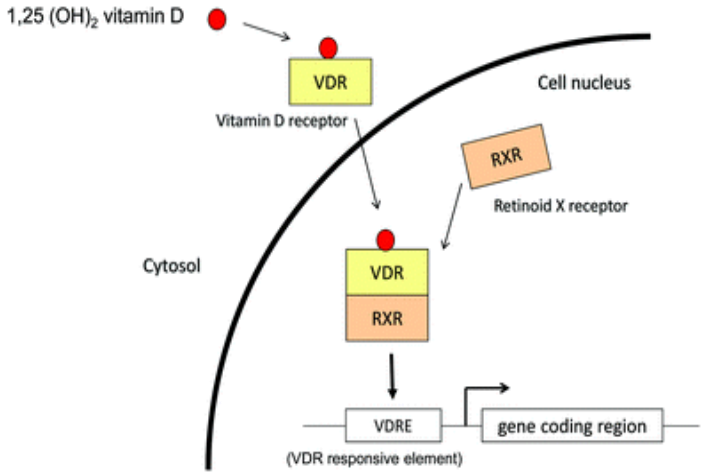


Figure 1-4. Vitamin D exerts biological roles via the VDR

The VDR is widely existing in a variety of cells and tissues, such as immunocytes, kidney, skeleton and skin. VDR is activated by binds to VD_3, and then heterodimerizes with a RXR. The heterodimer (VDR-RXR) can bind to VDRE of target genes to promoted or inhibit target gene expression levels [84]. $1,25(OH)_2D_3$-VDR affects cell proliferation, differentiation, growth, and biosynthesis, especially lipid biosynthetic pathways [85]. VD exerts biological roles by the VDR as shown in Figure 1-4.

1.2.5 VD and adipogenesis

Adipose tissue plays a vital biological function in animal systemic metabolism by storing and releasing energy and as an endocrine organ[76]. Obesity can lead to adipose tissue dysfunction, adipocyte hypertrophy and excessive accumulation of adipose tissue. VD deficiency is related to obesity and related metabolic diseases, obesity will change VD metabolic enzymes expression. VD is usually stored in skeletal muscle and adipose tissue, but adipose tissue is considered to be the main place for VD storage [86]. Meanwhile, VD can also regulate adipose formation, adipose tissue metabolism and endocrine functions. There results suggested that VD affects human obesity by regulating adipocyte differentiation and changing adipose tissue deposition. In adipose tissue, VD can regulate the development, and metabolism of adipocytes[87].

1.2.5.1 Effects of VD on adipogenesis

Adipogenesis is the process of stem cells differentiating into mature adipocytes. Briefly, after treatment with adipogenic stimulator, stem cells induce adipocyte specific gene expression and cell morphological changes, and then lipid droplets (in the form of triacylglycerol) were accumulated in cells. Previous studies showed that 1,25 (OH) $_2D_3$ had differential effects on adipogenic differentiation (**Figure 1-5)**. 1,25 (OH) $_2D_3$ reduces adipogenesis of 3T3-L1 cells[88], while increases adipocyte differentiation in adipose derived stem cells (ASCs) of human and mouse[89, 90], and in bone MSCs of mice and pigs[91, 92]. These inconsistent results may be caused by differences in adipogenesis programs between cell types.

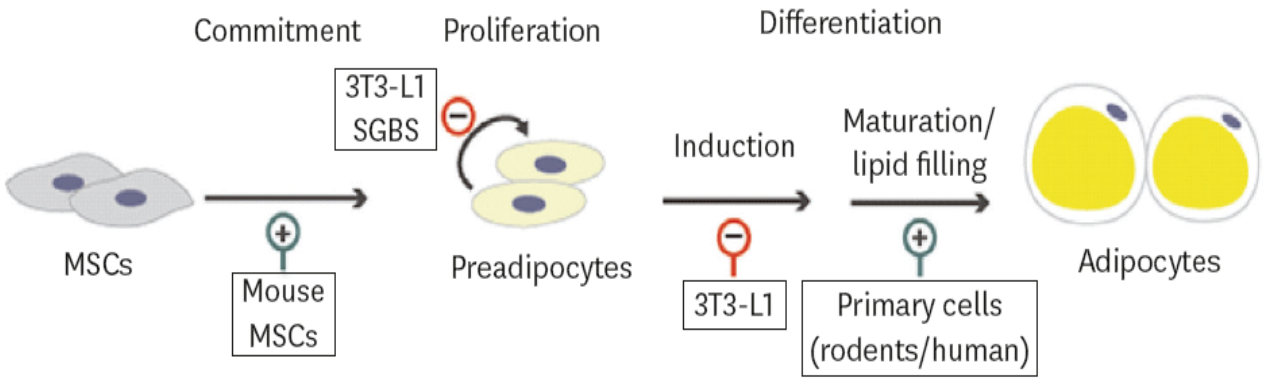


Figure 1-5. the effects of vitamin D on adipogenesis of different cell types[76]

1.2.5.2 Regulation of VD on adipogenesis

Adipose tissue expansion mainly includes the increase of adipocyte number (hyperplasia) and size (hypertrophy), which is correlated with obesity [93]. Mesodermal cells are regulated by various signals to form preadipocytes, and then preadipocytes differentiate into mature adipocytes through the regulation of intracellular signaling molecules [87].

Adipogenesis is a series of differentiation processes that resulting in adipocyte maturation. Adipocytes can influence many functions related to adipogenesis, such as fatty acid transfection, lipid synthesis, insulin signaling response, and adipokine secretion, and so on [94]. A large number of molecular interactions play an important role during adipogenesis, mainly including C/EBPβ and PPARγ expression [95]. In the early development stage of adipogenesis, C/EBPδ and C/EBPβ are mainly expressed and participate in the regulation of adipogenesis. The adipogenesis is improved by the biological regulation of C/EBPα, C/EBPβ, and C/EBPδ [96]. 1, 25$(OH)_2D_3$ can effectively inhibit the differentiation of 3T3-L1 preadipocyte by reducing the expression of C/EBPβ and PPARγ [97, 98]. Meanwhile, 1, 25$(OH)_2D_3$ also decreases fat accumulation and the expression of adipocyte lipid-binding protein 2 (AP2) of 3T3-L1 preadipocytes [99].

VD_3 is recognized as a potential regulator of adipogenesis, and it could affect adipogenesis of 3T3-L1 cells by decreasing LPL and FAS mRNA levels [100]. LPL is involved in the hydrolysis of triglycerides, and is a marker of adipocyte differentiation, which increases with the accumulation of TG [101]. In addition, VD_3 could inhibit differentiation and adipogenesis of 3T3-L1 preadipocytes by decreasing PPARγ, C/EBPα, FABP4 and stearoyl-CoA desaturase-1 (SCD1) expressions [102]. It also inhibited porcine preadipocyte differentiation by decreasing the expression of PPARγ and retinoid X receptor α (RXRα) mRNA [103]. These data indicated that VD_3 modulated adipogenesis and lipid metabolism in animals by altering adipogenic genes expression.

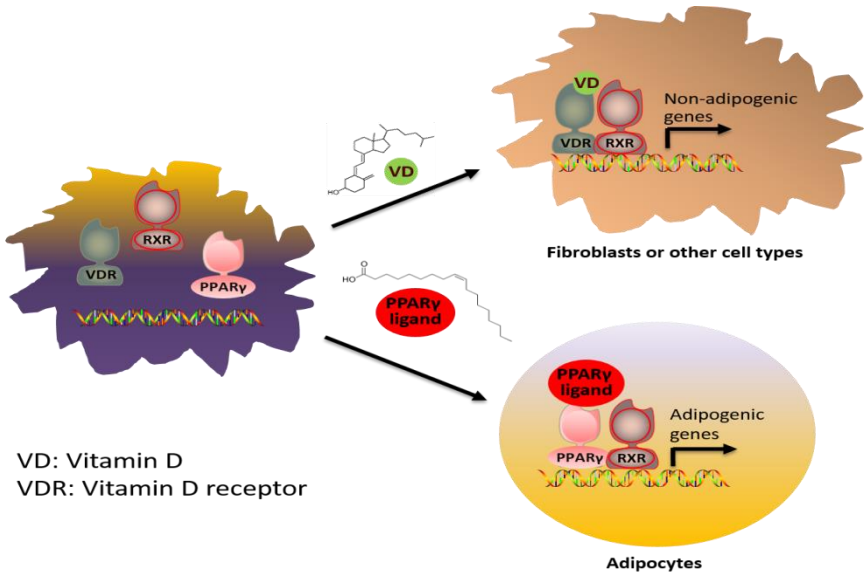


Figure 1-6. The relationship between PPARγ and VDR competition RXR [36]

Growing evidence shown that VDR plays a crucial biological function in adipogenesis, and VD and VDR are involved in the regulation of preadipocytes to adipocytes differentiation [88].

The biological function of VD is carried out by the action of $1,25(OH)_2D_3$-VDR, the target organs of VDR mainly includes kidney, liver, intestine, genitourinary tract, brain, bone, and immune cells [104, 105]. VDR is mainly expressed in the early period of adipocytes differentiation [106], VDR expression concentration is maintained by VD during adipocyte differentiation [88]. Knockdown of VDR of mice resulted in reducing fat mass, adipogenesis, and increasing β-oxidation rate [88, 106]. At different stage of adipogenesis, $1,25(OH)_2D_3$ can inhibit adipogenic activity by WNT/β-catenin signaling pathway and regulate mRNA expression and extracellular regulated kinase phosphorylation through mitogen activated protein pathway [98, 107].

Meanwhile, VDR gene polymorphism was positively correlated with obesity parameters [2]. The reason why VDR inhibits adipogenesis may be that RXR is a heterodimer partner of PPARγ and VDR, and it may compete with PPARγ for RXR, thereby reducing adipogenesis. Accordingly, VDR competes with PPARγ for RXR to regulate adipogenic differentiation[36]. As shown in Figure 1-6, the relationship between PPARγ and VDR competition RXR.

1.2.5.3 VD deficiency and adipogenesis

VD deficiency is usually defined as a serum level of 25-hydroxyvitamin D (25(OH)D) less than 20 ng/mL or 50 $nmol^{-1}$ [108, 109]. Obesity generally is defined as the excessive accumulation of body fat (adipose tissue), which exceeds the normal levels of a person's sex, age and body type [110]. Growing evidence showed that there is a certain correlation between obesity and VD deficiency, and the level of serum $25(OH)D_3$ is inversely correlated with obesity (excessive fat accumulation) and visceral fat [63, 111, 112]. Low serum 1, $25(OH)_2D_3$ concentrations are correlated with higher fat mass and body mass index (BMI).

Serum VD levels are negatively correlated with body fat [113, 114], and obese individual had lower serum VD levels [115, 116]. Recent study has found that VD deficiency during the perinatal stage can directly influence the expression of genes related to adipose tissue development in lean male mice offspring [62]. VD deficiency during pregnancy promoted the differentiation and proliferation of pre-adipocytes [117]. However another study observed that VD deficiency decreased adiposity in rats and caused altered expression of uncoupling proteins (Ucp1 and Ucp2) and steroid receptor coactivator 3 (SRC3), and dietary VD and calcium regulated adipose tissue function and metabolism[118]. Moreover, VD deficiency during pregnancy is also correlated to low birth weight and autoimmune diseases [119]. Lower maternal VD levels during pregnancy increases the risk of childhood obesity, and VD is also considered as a modifiable risk factor[120].

1.2.6 Role of vitamin D in adipose tissue

Excess energy can be stored in adipocytes of adipose tissue in the form of TG, and release it in the form of fatty acids and glycerol during fasting to meet the energy needs of the animals. Many adipokine such as adiponectin, leptin, interleukin (IL)-6 and resistin were secreted by adipocytes, which participated in endocrine function and energy balance. Consequently, adipose tissue, as a metabolic and endocrine organ, plays a key role in energy balance.

1.2.6.1 VD and adipokine secretion

Early studies indicated that VD regulates the secretion of adipokines [121, 122]. Adiponectin belongs to a serum protein which secreted through differentiated adipocytes, and there is a negative correlation between BMI and circulating adiponectin [123]. Previous study observed that the multimeric forms of adiponectin was down regulated in obese children with VD deficiency [121]. Whereas, VD_3 treatment had no effect on adiponectin expression in human adipocyte cultured in vitro [124]. Leptin has a positive correlation with body fat mass, and it can control lipid metabolism by inhibiting adipogenesis and stimulating lipolysis. The effects of VD_3 on the expression of leptin gene is tissue specific, and VD influences energy balance via directly regulating the expression of leptin [125]. The levels of serum leptin were lower in mice lacking 1, $25(OH)_2D_3$ and VDR knockout mice[126]. VD_3 can also stimulates the expression of leptin in mouse adipose tissue by upregulating VDR expression[122]. On the other hand, VD_3 suppressed the expression of leptin in 3T3-L1 adipocytes[127]. These results suggested that there is tissue specificity in the effect of VD_3 on leptin expression. Furthermore, leptin can block the inhibitory influence of VD on cell proliferation and adipogenesis in 3T3-L1 adipocytes[128].

$1,25\ (OH)_2D_3$ can inhibit many cytokines expression, and stimulate the secretion of adiponectin of 3T3-L1 adipocytes[129]. Whereas, another study found that $1,25(OH)_2D_3$ can reduce cytokines by the VDR of 3T3-L1 adipocytes[130]. Additionally, it also regulated the function of immune cells and macrophages of adipose tissue[131].

1.2.6.2 Effects of VD on adipose metabolic functions

VD involves in lipid utilization and metabolism in adipose tissue of animals, and VD_3 can increase the activity of lipoprotein lipase and its gene expression， while inhibit FAS in 3T3-L1 cells [132]. However, VDR can decrease lipid mobilization and utilization by inhibiting fatty acid β-oxidation process in WAT [106]. Previous results showed that lipid accumulation decreased with the increase of VD_3 dose. However, other study found that low-dose VD_3 stimulates the accumulation of TG, but high-dose VD_3 inhibits the accumulation of TG [133]. These inconsistent results suggested that VD_3

affect lipid metabolism in a dose-dependent manner. VD_3 increased PPARγ, LPL and AP2 expression of bone marrow stromal cells from pigs in dose-dependent manners [92]. GPDH (Glycerol-3-phosphate dehydrogenase) gene is usually used as a marker of adipogenesis, mainly expressed in mature adipocytes, and study found that VD also inhibited LPL and GPDH expression in the mature adipocytes [103]. The GPDH activity and lipid accumulation of porcine adipocytes were decreased by VD in a dose-dependent manner. Moreover, VD has a positive impact on adipogenesis of porcine MSCs, while a negative effect on porcine mature adipocyte.

The balance between lipid synthesis and lipolysis determines the amount of triacylglycerol stored in adipocytes. Previous report found that $1,25(OH)_2D_3$ regulates calcium entry into adipocytes and affects lipid metabolism by membrane-bound VDR. Meanwhile, $1,25(OH)_2D_3$ stimulated fatty acid oxidation, regulated lipid metabolism and glucose utilization, and decreased triacylglycerol accumulation and *de novo* lipogenesis of 3T3-L1 adipocytes [134]. $1,25(OH)_2D_3$ can also regulate the rate of fatty acid oxidation of 3T3-L1 adipocytes by stimulating several genes related to fatty acid oxidation [134, 135]. These findings suggested that $1,25(OH)_2D_3$ has catabolic function in adipocytes and can reduce lipid accumulation, which may decrease adipocytes' size. In addition, VD also regulates glucose metabolism and insulin actions of adipocytes. Emerging evidence shown that $1,25(OH)_2D_3$ increased glucose transport and insulin-stimulated AKT phosphorylation of 3T3-L1 adipocytes [129, 130].

1.2.6.3 Effects of VDR in adipose tissue on energy metabolism

VDR is expressed in the early stage of adipogenesis of 3T3-L1 cells, indicating that VDR signaling pathway plays a pivotal function in adipocyte biology and function[88]. Previous report found that VDR of mature adipocytes inhabit weight gain of mice, and adipocyte VDR signaling influence fat mass and body weight in female mice [136, 137]. These findings indicated that VDR also participates in energy metabolism in adipose tissue. VDR mediated secreted frizzled-related protein 2 (SFRP2) and Dikkopf 1 (DKK1) inhibited the adipogenic differentiation of murine bone marrow stromal cells (BMSCs), and VDR can partially inhibit adipogenesis of BMSCs by

decreasing the expression of typical Wnt signaling pathway[138]. VDR knockout mice decreased body weight, body fat, serum leptin and lipid, while increased calorimetric parameters. Meanwhile, VDR knockout mice has higher beta-oxidation and the carnitine palmitoyl transferase 2 (CPT2) expression in WAT [85, 106].

Taken together, VD plays a variety of physiological functions except from its classical function in keeping bone metabolism and calcium homeostasis. Emerging evidence indicated that VD deficiency is associated with obesity, adipose tissue is a main target for VD actions, and VDR expression is in adipose tissue. Moreover, VD_3 may decreases adipogenesis through inhibiting the expression of adipogenic transcriptional factors, and decreasing lipid accumulation of adipocyte. Meanwhile, the overexpression of VDR in 3T3-L1 adipocytes suppressed the differentiation efficiency of preadipocytes, and there is a certain correlation between VDR and the increase of human adiposity [2]. VD affects adipocyte formation and metabolic and endocrine functions of adipocytes tissue. The function of VD on adipose metabolism may be due to its regulation of lipolysis and lipid synthesis and improving insulin signaling pathway [76]. However, the function of VDR in lipid metabolism remains controversial, because VDR-null mice had decreased fat mass and had the characteristics of lean phenotype.

1.3 Effects of maternal nutrition on adipose tissue and skeletal muscle development in offspring

During pregnancy, the development of embryo/fetus depends entirely on the supply of nutrients and the clearance of metabolic by-products through the maternal organism. Therefore, the continuous lack of nutrition supply may have serious consequences for fetal development and may be maintained until adulthood[139]. Therefore, there is a correlation between prenatal development and the phenotypic appearance of muscle and fat after birth, and the phenotypic development of muscle and fat in offspring can be regulated by maternal nutritional status. Moreover, compared with the fattening period, improving the physiological and nutritional

conditions of the fetus, postnatal and early weaning period will have an important impact on adipogenesis[36].

Growing evidence demonstrated that maternal nutrients fluctuations during gestation impacted foetal development, growth performance and meat quality in offspring [7, 140]. Maternal malnutrition leads to inadequate nutrition supply to the fetus and has a negative impact on fetal development [141]. In addition, maternal nutrient restriction influences physiological characteristics of skeletal muscle in offspring. Fetal nutrient deficiency is mainly caused by malnutrition during pregnancy, and maternal nutritional deficiency has an important effect on the development of adipose tissue and skeletal muscle in offspring[142].

1.3.1 Fetal programming of adipose tissue

The fetal development of adipose tissue occurs in a metabolic and hormonal environment. In this environment, the goal of the fetus is to maximize its growth potential, which mainly depends on its nutritional, genetic, and spatial constraints. Adipose tissue is one of the last deposited tissues in fetal development, and its quantity is very sensitive to the nutritional constraints during pregnancy period [143]. After birth, animal adipose tissue can be used as an endogenous energy storage (WAT), and as a basic source of heat (brown adipose tissue or beige adipose tissue). Compared with fattening period, the nutritional and physiological status of fetus, postpartum and early weaning period have a greater impact on adipogenesis [36].

Majority of adipocytes are produced in the fetal period and early postnatal stage, while the hyperplasia of adipocytes in perirenal fat basically stops after birth[144]. Although new adipocytes can be produced for life, their ability will gradually weaken with the increase of animal age [18]. Fetal programming determines the number of adipocytes in offspring. Adipose tissue development depends on preadipocyte proliferation, adipocyte hypertrophy and angiogenesis, and it can be divided into the stages of determination and differentiation. Adipocyte progenitors are originated from mesoderm, which is called FAPs, and most of them are formed during fetal development [24, 145]. During postnatal development, some FAPs exist in the stromal vascular part of fat tissue, which is the source of adipocytes and preadipocytes in the

later development of adipose tissue [7, 14]. Adipogenic differentiation and commitment stages are mainly regulated by PPARγ and zpf423, respectively. Meanwhile, the development of adipose tissue is closely related to the development of capillary network.

In addition, there is a temporal and spatial correlation between adipogenic differentiation of stem cells and angiogenesis, which often occurs with the formation of capillaries. Blood vessels can be used as a progenitor cell pool for adipocyte proliferation and provide essential transcription growth factors for adipogenic differentiation of progenitor cells. Therefore, the expression of genes encoding angiogenesis regulatory factors reflects the development of adipose tissue in fetuses and newborns. The number of adipocytes is determined by proximity to entrance of the great arterioles [146]. Previous study observed that there is a correlation between the development of intramuscular adipocytes and vascular development or blood flow[147].

Adipose hyperplasia is related to the number and size of adipocytes in animal fattening stage. Additionally, the development speed of adipose tissue in different parts of animals is different, and the development of IMF in pigs is later than that in other parts. The development of intramuscular fat and subcutaneous fat in pigs is relatively slow, while the growth of perirenal fat is rapid. The deposition of porcine adipose tissue is mainly in the late stage of growth and development (after about 80 kg body weight). In the early stage of growth and development, the deposition of porcine adipose tissue is mainly the increase of the number of adipocytes, while in the later stage of growth and development, it is mainly the increase of the volume of adipocytes.

1.3.2 Maternal nutrition and adipose tissue development in offspring

The effect of mother on fetal or offspring fat deposition depends largely on nutritional intervention during pregnancy. Maternal nutrient restriction suppressed angiogenesis during fetal development, and decreased adipogenesis of offspring. Previous reports showed that adipocytes mainly occur from mid-gestation to early weaning periods (Figure 1-7). So, maternal nutrient status during pregnancy can affects adipocyte hyperplasia, that regulating the tissue development of adipose and IMF

content [14]. The main stages of porcine adipocyte differentiation are embryonic stage and early postnatal stage.

Moreover, adipose tissue growth is the result of hyperplasia and hypertrophy from mid gestation, the deposition of perirenal adipose tissue in fetal lambs is sensitive to maternal nutrition and placental nutrient exchange capacity. Due to maternal malnutrition, fetal glucose supply is insufficient, which greatly changes the fetal endocrine environment and has a significant impact on adipose tissue maturation[143]. The offspring from female rats fed low protein during gestation period decreased visceral WAT mass, changed fat distribution, and enhanced the proportion of small adipocytes in epididymal WAT by reducing the levels of key insulin signaling protein[148]. These results suggested that maternal protein deficiency during gestation and lactation changed the distribution and morphology of WAT in the offspring. Decreasing maternal nutrition during late pregnancy can lead to smaller fat depots, lower plasma insulin and glucose of offspring[143, 149]. In addition, Marchix and his colleagues found that maternal linoleic acid supplementation regulated adipose tissue homeostasis in young offspring rats [150].

Taken together, according to the existing research results, maternal pregnancy is the best window period to regulate adipogenesis in offspring. Therefore, scientifically adjusting the maternal nutrition status during pregnancy may be an effective way to improve the meat quality and growth performance of offspring.

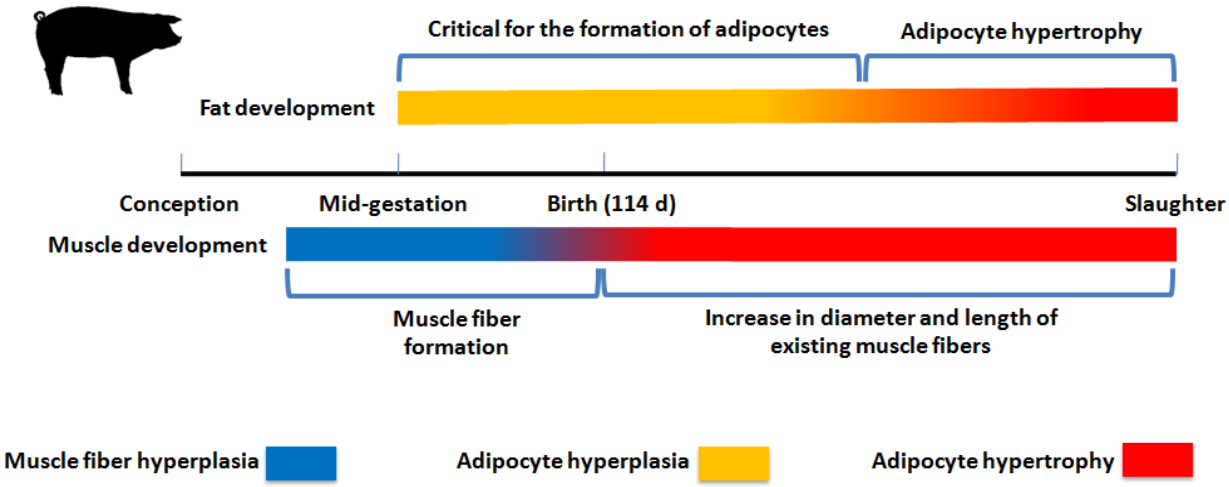


Figure 1-7 Approximate timelines for skeletal muscle and adipose tissue development of pigs

Maternal VD deficiency in gestation has been a widespread all over the world, and it has a long-term impact on adiposity in offspring. Previous finding demonstrated that maternal VD deficiency during pregnancy didn't affect the WAT mean adipocyte size, while increased the expression of VDR and PPARγ, and affected adipose development of lean male mice offspring, which indicated that maternal VD levels influence adipogenesis in offspring mice[62]. Another study found that maternal VD deficiency during pregnancy improved the differentiation and proliferation in preadipocytes, increased body fat, and resulted in the obesity of offspring mice, which suggested that maternal VD status during pregnancy can affect adipogenesis and lipid metabolism of offspring mice[117]. In human study, maternal VD deficiency or insufficiency during pregnancy increased fat mass, the risk overweight in offspring before and early after birth[151, 152]. Maternal VD concentrations in low dose during pregnancy increased the risk of obesity and body mass index in offspring[120]. Maternal VD insufficiency during gestation decreased post-weaning body weight, and influenced energy metabolism in offspring male mice, and there was no significant in female offspring mice, which indicated that maternal VD deficiency during gestation affects energy homeostasis and adipose tissue metabolism in offspring mice in a gender dependent manner [153]. Maternal VD inadequacy during mid gestation increased abdominal subcutaneous adipose tissue volume in offspring, which suggested that there is a relationship between maternal VD status and neonatal abdominal adiposity [154]. Maternal VD supplementation in low dose increased infant weight, and play a role in infant adiposity [155].

However, several studies found that maternal VD status did not affect adipose tissue development, and adipogenesis in offspring rats, which indicated that maternal VD levels did not play a fundamental role in fetal adipogenesis [156], and fetal VD levels aren't related to growth and adiposity of infancy [157].

1.3.3 Fetal programming of skeletal muscle development

Limited studies have showed that skeletal muscle cells, fibroblasts and adipocytes are originated from the common mesoderm MSCs, and there is a competitive relationship between them. Developmental biology shows that most MSCs

enter the skeletal muscle development process in the early stage of embryonic development, while only a small number of MSCs enter the adipose tissue development in the middle stage of embryonic development [7, 14]. So, skeletal muscle cells are occurred during the early gestation, but adipocyte progenitors are generated during mid-gestation.

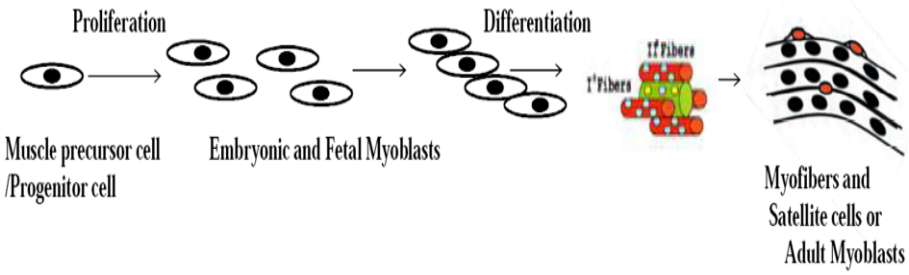


Figure 1-8 The different period of myofibers formation[158]

The early development of skeletal muscle mainly includes primary and secondary myogenesis. Among them, primary myofibers first occurred in the embryonic period, and then the secondary myofibers are formed that account for most adult muscle fibers [159]. The different period of myofibers formation is shown in Figure 1-8, and embryonic, fetal and adult myoblasts appear sequentially during myogenesis [158].

The muscle growth of postpartum animals is due to the increase of muscle fiber size rather than the formation of new muscle fiber [160]. In addition, satellite cells are located around mature muscle fibers in postnatal muscles, which are very important for postnatal muscle growth [161]. Muscle fiber formation is mainly formed during the fetal period, so this state of muscle development is easily affected by maternal nutritional status. At this stage, the reduction of muscle fiber formation will reduce the number and mass of muscle fiber, which will have a negative impact on the growth performance of offspring [142]. Previous study observed that $1.25(OH)_2$ VD_3 improves myogenic differentiation via reducing cell proliferation, adjusting myostatin and pro-myogenic growth factors expression of C2C12 skeletal muscle cells [162], which indicated that VD is also an important regulator of myogenesis.

1.3.4 Maternal nutrition and muscle tissue development in offspring

The number of muscle fibers in muscle largely depends on pregnancy[163], which has a vital effect on muscle growth and development in the later stage of life. Therefore, appropriate animal nutrition during pregnancy can improve the production performance, muscle development and meat quality of offspring. Pregnancy is very crucial role for skeletal muscle development, and the amount of muscle fiber in adults is determined in the womb. Meanwhile, nutrition deficiency during mid-pregnancy decreased the numbers of muscle fiber, while nutrition deficiency during late pregnancy decreased the size of muscle fiber in offspring[50]. Therefore, the dietary restriction of the mother will lead to the damage of muscle development during pregnancy, which affecting the number and composition of fibers in offspring [142]. Nowadays, there are more and more studies on maternal nutrition during pregnancy regulating muscle development, changes in muscle fiber types and related gene expression of offspring. Such as, maternal poor nutrition influenced the number of fast fibers, total number of fibers and carcass composition in offspring lamb [164]. In addition, maternal nutrition insufficiency during gestation decreased skeletal muscle development, while increased type IIb myofibers in offspring with long-term manners, which indicated that maternal nutrient status influenced the properties of skeletal muscle in offspring[142]. Maternal selenium addition also increased breast muscle yield in offspring broiler by enhancing the expression of Myf5, MyoD and SeIW, which suggested that maternal selenium status influenced skeletal muscle development in offspring [165]. Maternal chromium restriction damaged muscle development and glucose uptake in muscle of offspring rats, which indicated that maternal dietary chromium levels also affect skeletal muscle development and function in offspring[166]. Additionally, maternal protein restriction can regulate muscle fibers in offspring rat, and this effect is lasting and irreversible[167]. In recent years, with the deepening of research on the regulation animal muscle development of vitamin A and VD, the effects of maternal vitamin A or vitamin D levels during pregnancy on the muscle development in offspring has gradually attracted researchers' attention.

Taken together, maternal nutrition deficiency or insufficiency during pregnancy can suppress the number of muscle fibers, and decrease muscle mass, which affects animal performance.

Emerging evidence found that VD_3 plays a regulatory function in the fetal muscle development in animals[61, 168, 169]. Meanwhile, muscle growth potential in animals is positively correlated with the total number of muscle fibers at the fetal stage [170, 171]. These findings suggested that VD_3 may affect postnatal muscle development and meat quality in animals by improving muscle fiber number during the fetal period. Previous research demonstrated that nutrient status of mothers influences fetal development and has a long-term impact on the postnatal health in offspring, and the early to mid-gestation stage is very critical for skeletal muscle development[142]. Meanwhile, nutrient restriction from early to mid-gestation decreased the number of fetal skeletal muscle fibers[141]. Maternal VD addition with 25-hydroxycholecalciferol increased porcine fetal skeletal muscle development and myoblast activity[169]. Additionally, maternal VD insufficiency results in smaller muscle fibers, and decreases the expression of muscle cell development genes, which suggested that maternal VD insufficiency has an important effect on gene expression profile and morphology of skeletal muscle of newborns[172]. In addition, VD also has important direct effects on skeletal muscle[173], and improving maternal VD levels with $25OHD_3$ can facilitate the development of prenatal skeletal muscle in offspring pig by regulating the expression of muscle transcription factors[174]. Maternal VD_3 and $25(OH)D_2$ supplementation accelerated the primary muscle fiber of offspring pigs at birth, but had no effect on the muscle fiber of offspring pigs at weaning[175].

Overall, Prenatal adipogenesis and muscle development are influenced by maternal nutrition, and maternal nutrition during pregnancy has a long-term impact on adipogenesis and muscle growth and development. Therefore, Proper maternal nutritional regulation during pregnancy can improve adipogenesis and the development of fetal skeletal muscle, and then improve the marbling and meat quality of progeny.

1.4 Animal meat quality function, technology properties and its influencing factors

Meat is the main source of protein of human beings. Excessive consumption of meat (meat products) usually leads to increased risk of overweight, obesity and chronic diseases[176]. Meanwhile, meat quality is usually associated with human health and repeat purchases of consumers, mainly included color, WHC, cooking loss, flavor, tenderness, and juiciness. Previous reports have been demonstrated that tenderness, juiciness, and flavor are the most important meat characteristics, which can be regulated by nutrition, mainly through the influence of nutrition on the quantity and type of fat in meat [177, 178].

1.4.1 Meat quality technology properties

1.4.1.1 IMF

The content of IMF is correlated with meat palatability[18, 179]. Fetal and neonatal stage is the key period to produce animal intramuscular adipocytes [20], and they generate marbling at the fattening period in offspring. Previous study found that the reasonable amount of IMF improved the palatability, taste, juiciness, flavor, tenderness, and quality of meat[180, 181]. The pork quality is usually affected by flavor, tenderness and juiciness[182, 183].

1.4.1.2 Tenderness

Meat tenderness is usually affected by the myofibrillar effects and the presence and cross-linking of connective tissue. It was observed that tender meat contains more IMF and less connective tissue, and there is a positive correlation between IMF content and meat tenderness[184, 185].

1.4.1.3 Juiciness

The juiciness of meat is often influenced by raw meat quality and cooking procedure. Meanwhile, the juiciness of meat is correlated to pH value and IMF content of pork. It was previously noted that higher content of IMF is associated with better meat quality[186, 187].

1.4.1.4 Meat color

Meat color is usually used for assessing freshness and meat quality attributes, which is the direct factor that determine consumers' purchase [188, 189]. a*, b* and L* values of meat color represents redness, yellowness, and lightness, respectively [190]. Previous study has demonstrated that pork color discoloration is correlated to pigment and lipid oxidation [191].

1.4.1.5 Cooking loss

Cooking loss is generally considered to be the release of chemically bound water due to fat melting and protein denaturation during cooking [192]. There is a negative correlation between cooking loss and eating quality of meat[182].

1.4.1.6 Shear force

Shear force usually reflects the tenderness of meat, and the increase in tenderness is associated to the length of frozen storage[193], and it was previously demonstrated that shear force is negatively correlated to IMF content of muscle[185].

1.4.1.7 Thawing loss

Freezing and thawing usually influenced the amount of thawing loss and drip loss, and when the freezing time was more than 19.5 min, the amount of thawing loss and drip loss was significantly higher than that before freezing[193]. It was demonstrated that thawing loss usually affects the color and sensory quality of meat[194], and is associated with the destruction of muscle fiber structure and the denaturation of protein[193].

1.4.1.8 Low field nuclear magnetic resonance (LF-NMR)

LF-NMR can represent the migration and distribution of water in meat, and it is helpful to understand the influence of postmortem process, cooling, and other factors on WHC of meat. Several studies have reported that the LF-NMR relaxation times reflects the degree of tightness between water and substrate, which could distinguish water interacting with macromolecules, water in myofibrils and reticular tissue, as well

as extracellular water in meat[195]. T2a corresponds to the bound water, T21 represents the immobilized water, T22 corresponds to the free water[196].

1.4.1.9 WHC

WHC affects economic and sensory properties of meat, which is very important in the meat industry[197]. Previous studies showed that the WHC is very important for reducing exudates, and improving palatability, juiciness, tenderness, and meat quality attributes[197, 198].

1.4.1.10 pH value

Previous study found that the decrease in pH value is due to the accumulation of lactic acid produced by anaerobic glycolysis[199]. The decline in pH in meat samples affects WHC, moisture, tenders, and color of meat.

1.4.2 VD and meat quality function

In previous reports it was also observed that dietary VD_3 level affects pork quality by regulating shear force, pH value and meat color[200, 201]. In other studies it was found that short-term feeding with VD_3 can improve meat color, but does not change the tenderness of pork-loin chops[202]. Such results revealed that dietary VD_3 supplementation could improve pork quality. Previous study also found that VD_3 increased tenderness of *longissimus* muscle in beef through activation of m-calpain[203]. Dietary supplementation with VD_3 (0.5 million IU/steer per day) for 8 days before slaughter can improve tenderness in steaks from different primal cuts[204]. Meanwhile, dietary addition with 1alpha-OH D_3 increased yellowness and lightness of the thigh and breast meat, but it reduced the WHC and shear force of the thigh meat[205]. Feeding supra nutritional levels of VD_3 (40,000 IU of VD_3/kg of feed) for at least 44 d regulated pork pH and color, which improving pork quality[200]. At 7 and 14 d of shelf storage, VD_3 addition results in higher a* values and lower L* values in loin chops[202].

1.4.3 Maternal nutrition and meat quality of offspring

Maternal nutrition status during pregnancy has a long-term effect on meat quality and growth performance in offspring, which may be correlated to the changes in fetal

programming of adipose tissue and skeletal muscle development. In pig study, Previous results showed that maternal nutrition supplementation during mid-gestation increased cross-sectional areas, while decreased the number of muscle fibers and lightness in offspring pigs, which indicated that increased sow nutrition influenced the development of muscle fiber and meat quality in offspring pigs[206].

Maternal methionine supplementation increased lean percentage, pH value 24 h postmortem, and the expression of myosin heavy chin IIX and myogenic differentiation and muscle regulatory factor 4, which revealed that maternal methionine status affects carcass characteristics and meat quality in offspring pig[207]. Meanwhile, maternal VD_3 supplementation during pregnancy increased hot carcass weight, carcass yield percentage, while decreased loin depth and back fat thickness in offspring pigs, which suggested that maternal VD supplementation during gestation improved growth performance and carcass characteristics in offspring[61].

1.5 Research purpose and plan of this experiment

1.5.1 Research purpose of this experiment

Pork quality is associated with human health, the distribution, composition, and deposition of adipose tissue are the crucial factors affecting pork quality. In particular, the content of IMF in pigs mainly depends on the number of precursor adipocytes and the ability of fat synthesis and accumulation. Developmental biology has shown that porcine adipose tissue and skeletal muscle are derived from the directional differentiation of embryonic mesodermal MSCs.

The proportion of MSCs differentiated into adipocytes plays a vital role in the formation of IMF in fattening pigs at later stage. In addition, IMF deposition in pigs is usually affected by many factors, including genetic background, nutrition, external environment and feeding methods. Maternal nutrition status during pregnancy has a long-term impact on growth performance, adipogenesis, muscle development, and meat quality in offspring.

Recent studies found that VD_3 is an important nutritional regulator of adipogenesis, and it plays an important regulatory role in the anabolism of animal

adipocytes. Although, VD_3 is involved in the regulation of fat accumulation and lipid metabolism by regulating adipogenic gene expression, reports about the role of maternal VD_3 status during pregnancy in formation of functional and technological properties of pork in offspring pigs are still unclear.

The purpose of this study was to investigate the effects of maternal VD_3 during pregnancy on the formation of functional and technological properties of pork in offspring pigs. In the present study, pregnant sows with skeletal muscle and adipogenesis overlapping period (41st gestational age) were selected as experimental animals to investigate the effect of different maternal VD_3 levels on meat quality function, processing performance, and technological properties in offspring pigs by feeding trial, slaughter trial and technique related to meat quality function and technological characteristics. The results will provide a scientific theoretical basis and implementation plan for the early nutritional regulation of pork quality during fetal period by regulating maternal dietary VD_3 status.

1.5.2 Research plan of this experiment

The main research plan of this experiment is as follows:

1. The study of the effect of VD3 on the potential food factor of pork
2. Research of physical-chemical indicators of meat quality and its ability to be stored
3. Recommendations for improving the technology of meat production with given functional and technological properties

The technology route of this dissertation is shown in Figure1-9.

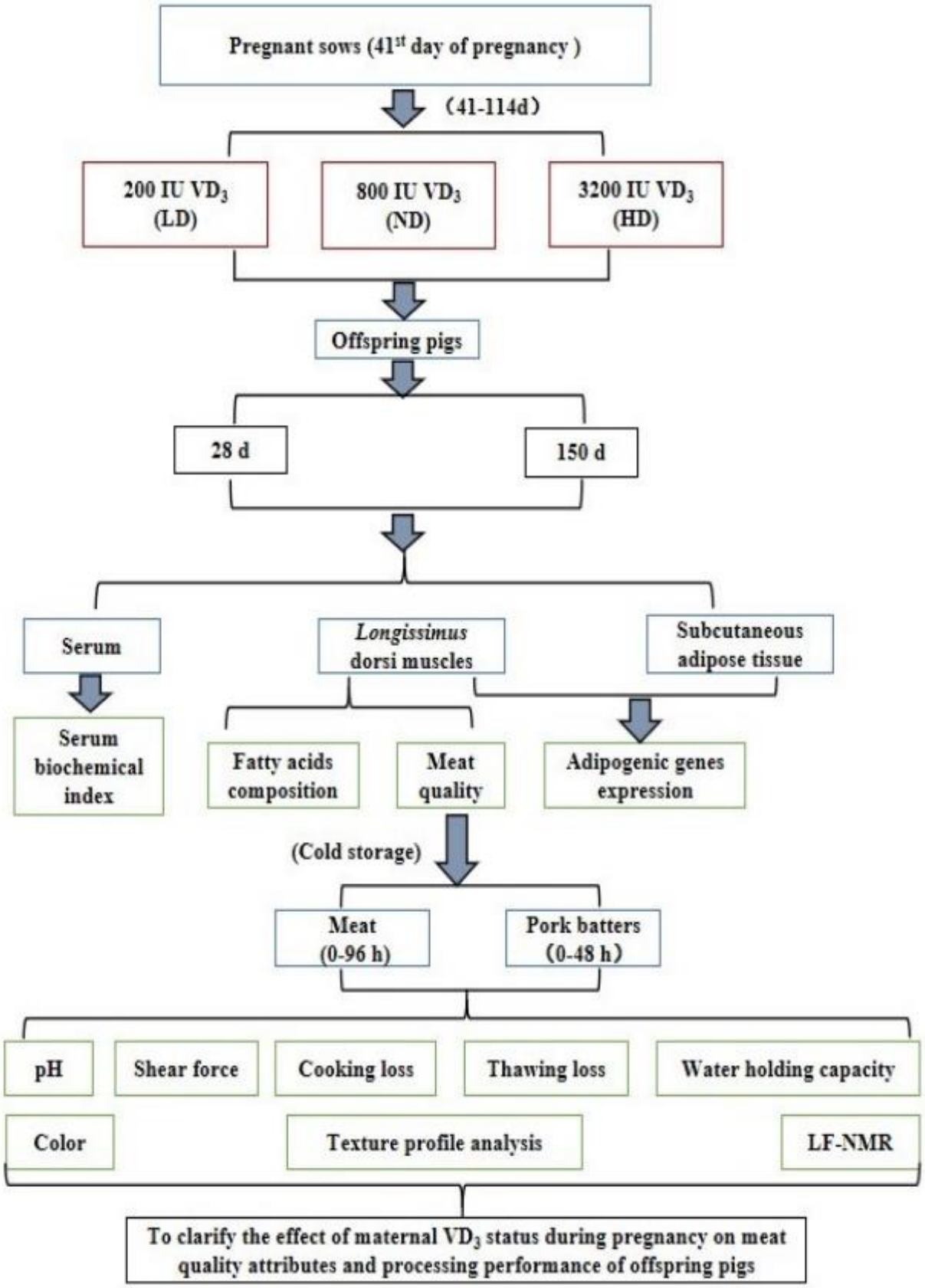


Figure 1-9 Technology roadmap of the dissertation

Conclusion in section 1

1. VD_3 is recognized as a regulatory factor affecting the formation of functional and technological properties of pork.
2. Maternal VD_3 nutrition levels during pregnancy has a long-term impact on meat quality function and technology properties in offspring.
3. The purpose of this study was to investigate the formation of functional and technological properties of pork by the influence of vitamin D_3 on the differential maternal level of pigs.

SECTION 2 OBJECTS AND SUBJECTS OF METHODS

2.1 Materials of research

In the theoretical and experimental work, VD_3, pregnant sows (41st days of pregnancy) and their offspring pigs were used as experimental research objects to study the effects of different levels of VD_3 supplementation during pregnancy on lipid metabolism and meat quality characteristics of offspring pigs.

In the study, VD_3 (feed grade) was characterized by 500, 000 IU per gram, and was provided by Zhejiang Garden Biochemical High-Tech Co., Ltd (Hangzhou, China).

In addition, all diets were formulated to meet or exceed the national research council (NRC, 2012) recommendations, and shown in Table 2-1, Table 2-2**,** and Table 2-3, respectively.

Table 2-1 Gestation diet composition of sow[1]

Ingredient %		Nutrients[3]	
Corn	61.91	DE, MJ/kg	13.03
Wheat bran	16	CP, %	16.45
Soybean meal	19	Ca, %	0.68
Fish meal	0	Available P, %	0.36
Limestone	1.5	Lys, %	1.04
$CaHPO_4$	0.29	Met, %	0.24
Salt	0.3	Met+Cys, %	0.52
Premix[2]	1		
Total	100		

[1]Gestation diet for low VD_3 (LD), normal VD_3 (ND) and high VD_3 (HD) groups from 41 days of age until birth. Their compositions were similar except VD_3 levels.

[2]Provided the following (unit/kg): 10 mg of Cu, 80 mg of Fe, 25 mg of Mn, 100 mg of Zn, 0.2 mg of I and 0.2 mg of Se. 4000 IU of vitamin A, 200 IU of VD_3 (LD group), 800 IU of VD_3 (ND group), 3200 IU of VD_3 (HD group), 44 IU of vitamin E, 1.0 mg of vitamin K_3, 1 mg of vitamin B_1, 3.75 mg of riboflavin, 1 mg of vitamin B_6, 15 mg of vitamin B_{12}, 12 mg of pantothenic acid, 10 mg of niacin and 1.25 mg of choline.

[3]All data were analyzed values except digestible energy, which was calculated using swine National Research Council (NRC) (2012) values.

DE, digestible energy. CP, crude protein. Lys, lysine. Met, methionine. Cys, cystine.

Table 2-2 Lactation diet composition of sow[1]

Ingredient %		Nutrients[3]	
Corn	68	DE, MJ/kg	13.42
Wheat bran	8.02	CP, %	16.77
Soybean meal	20	Ca, %	0.70
Fish meal	1	Available P, %	0.36
Limestone	1.5	Lys, %	1.09
$CaHPO_4$	0.18	Met, %	0.27
Salt	0.3	Met+Cys, %	0.54
Premix[2]	1		
Total	100		

[1] Lactation diets with the same VD_3 levels were fed lactating sows in LD, ND and HD groups, and their offspring piglets were weaned 28 days of age.

[2]Provided the following (unit/kg): 20 mg of Cu, 80 mg of Fe, 25 mg of Mn, 100 mg of Zn, 0.2 mg of I and 0.2 mg of Se. 2000 IU of vitamin A, 800 IU of VD_3, 44 IU of vitamin E, 1.0 mg of vitamin K_3, 1 mg of vitamin B_1, 3.75 mg of riboflavin, 1 mg of vitamin B_6, 15 mg of vitamin B_{12}, 12 mg of pantothenic acid, 10 mg of niacin and 1 mg of choline.

[3]All data were analyzed values except digestible energy, which was calculated using swine National Research Council (NRC) (2012) values.

Table 2-3 Ingredients and nutrients of basal experiment diets of offspring pigs

Item	28-90 days	91-150 days
Ingredient %		
Corn	71.95	76.5
soybean	24	20
Limestone	0.7	0.9
CaHPO4	1.7	1.2
Lysine	0.25	0.21

Continuation of Table 2-3

Item	28-90 days	91-150 days
Salt	0.4	0.4
Premix[1]	1	1
Total	100	100
Nutrients[2]		
DE, MJ/kg	13.75	13.79
CP, %	17.78	15.65
Ca, %	0.71	0.67
Available P, %	0.42	0.35
Lys, %	0.96	1.11
Met, %	0.27	0.26
Met+Cys, %	0.55	0.52

[1] Provided the following (unit/kg): 10 mg of Cu, 80 mg of Fe, 30 mg of Mn, 80 mg of Zn, 0.5 mg of I and 0.3 mg of Se. 5850 IU of vitamin A, 1251 IU of VD_3, 20 IU of vitamin E, 1.86 mg of vitamin K_3, 3 mg of vitamin B_1, 3.6 mg of riboflavin, 1.5 mg of vitamin B_6, 20 mg of vitamin B_{12}, 18 mg of pantothenic acid, 26 mg of niacin and 56 mg of choline.

[2] All data were analyzed values except digestible energy, which was calculated using swine National Research Council (NRC, 2012) values.

Objects of the study - Formation of functional and technological properties of pork by the influence of Vitamin D_3 on the differential maternal level of pigs.

The subject of the study Pork meat, functional and technological properties of meat, differential maternal status of VD_3 sow, offspring.

2.2 Research Methods

2.2.1 Experimental Design

All pigs handling protocols in this study were approved by the Animal Care and Use Committee of Henan Institute Science and Technology (Xinxiang, P.R. China). A total of 27 pregnant sows (41st days of pregnancy) with the same parities and similar body weights (144.62 ± 2.3kg) were randomly divided into low VD_3 (LD), normal VD_3

(ND) and high VD_3 (HD) groups, each group includes 3 replicates with 3 sows per replicate, which were fed 200, 800 and 3200 IU of VD_3/kg basal diet, respectively. And maintained on these diets throughout pregnancy of sows up until birth. During d 41 to 110 of gestation, sows were housed in gestation stalls (2.10×0.55 m), and fed 2.2 kg/d of the gestation diets per sow. On d 110, sows were transported to the farrowing house and were housed in farrowing crates (2.20×0.60 m for sow, and 2.20×1.2 m for piglets). Every farrowing crate was equipped with a single feeder and nipple water. From birth to weaning (28 days of life), all lactating sows were switched to the ND diets. The first 7 d after farrowing, feed were increased gradually according to sows' appetite. After d 7, all sows were allowed *ad libitum* access to the lactation diet, and water via nipple drinkers. After farrowing, all piglets only obtain nutrition through breast milk without adding feed during lactation.

All offspring pigs used in the experiment were provided with *ad libitum* access to feed and water. From birth, 72 offspring piglets (sex balanced) from 348 offspring born to 27 sows were allotted into 3 groups (every group containing 3 replicates with 8 offspring per replicate) again according to their maternal VD_3 supplementation group. Offspring pigs from all groups were fed the same VD_3 level diet, and was housed and fed under the same condition. At 150 days of age, six offspring in each group (sex balanced, 2 offspring pigs per replicate) were selected to weighed and sent for tissue collection according to similar weights. During this period, all piglets were reared in the same condition, and had *ad libitum* access to an experimental diet and water via nipple drinkers.

2.2.2 Slaughter and samples collection

From birth, a total of 72 piglets (sex balance) from all their 348 offspring were allotted into 3 groups again according their mother's gestational group. Each group has 3 replicates with 8 offspring (sex balance) per replicate. Briefly, at birth, all piglets in each group (from 9 litters) were weighed individually and ear tagged for identification. 24 piglets were randomly selected form each group (sex balance) according to the similar average birth weight, and the selected piglets (possibly from different litters) still stayed in their original litter until weaning. At their predesignated slaughter age

(28 and 150 days of life), 6 offspring with similar average body weight in each group (2 offspring per replicate, sex balance) were randomly selected to weigh and slaughter for tissue collection according to the method described by previous study [208].

In order to investigate effects of maternal VD_3 concentration during pregnancy on factors related to the formation of meat quality function in offspring piglets. At 28 days of age, the experimental pigs selected were electrically stunned, exsanguinated, dehaired and eviscerated after fasting 12 h. The head was removed and the carcass was split longitudinally, the *longissimus dorsi* muscle was quickly dissected and frozen in liquid nitrogen, and then stored at -80℃ until extraction for total RNA. Samples of blood were collected from 18 offspring (6 pigs each group according to average body weight), and allowed to clot overnight at 4℃. Serum was harvested following centrifugation (3,000 g for 10 min, at 4℃) and stored at -80℃ until analysis [56].

In order to investigate effects of maternal VD_3 during pregnancy on the formation of meat quality function and its regulatory factors in offspring pigs. At 150 days of age, the subcutaneous adipose tissue and *longissimus* dorsal muscle was quickly dissected and frozen in liquid nitrogen, and then stored at -80℃ until extraction for total RNA. Meanwhile, left half-carcasses without head, legs, and guts (except kidney) were weighed. Adipose and muscle tissue in the left half-carcass was dissected and weighed, the carcass fat content and carcass dressing percentage were calculated. The average backfat thickness (ABFT) was taken in the midline with a sliding caliper, and the average of three backfat thickness, measured on the first rib, last rib and last lumbar vertebrae. The analysis of IMF in the *longissimus* dorsal muscle was measured according to the AOAC (1990) procedures.

In order to investigate effects of maternal VD_3 during pregnancy on meat quality and fatty acids composition in offspring pigs. the *longissimus* muscle was separated from the left half-carcasses after an overnight (12 h) chill at 4℃. *Longissimus* muscle area of offspring pigs was measured using a planimeter by tracing its surface area at the 10th-rib (Planix 5.6, Tamaya Digital Planimeter, Tamaya Tecnics Inc., Tokyo, Japan). After cooling at 4℃ for 12 h, the shear force of *longissimus* muscle was measured according to the method of previously report [209]. The muscle sample was put

into a water bath at 80℃ for 30 min until the central temperature reached approximately 72℃, and then it was removed and cooled to a central temperature of about 4℃ for shear force analysis. The shear force of raw meat was defined as the arithmetic mean value of the maximum forces of 10 cylinders (3 cm in diameter, 4 cm in long), after discarding records which differ from the mean value of 10 record by more than 2 standard deviations. All the shear force were measured without freezing the meat in advance. The *longissimus* muscle samples were stored at 4℃ over 24 h (from 24 to 48 h after slaughter), and the drip loss of meat samples (3 cm in length, 1.5 cm in width and 1.5 cm in height) of offspring pigs was calculated according to the percentage weight ratio of meat sample before and after cold storage. The subjective color was determined using color standards (7-color discs), which ranged from 1 (greyish-white) to 7 (deep red). Marbling color scores (National Pork Producers Council Standards, NPPC, 2000) were determined by color standards (5-color discs), which ranged from 1 (pale pinkish to grey) to 5 (dark purplish to red). The subjective color and marbling color scores were determined at approximately 48 h post-mortem.

In order to investigate effects of maternal VD_3 status on quality traits of *longissimus dorsi* muscle in offspring pigs during postmortem storage. After chilled at 4℃ for 24 h, *longissimus dorsi* muscles were removed from carcasses and assigned into 3 parts. Three postmortem times of *longissimus dorsi* muscles were 24, 48, and 96 hours, respectively. The *longissimus dorsi* muscles were individually place on the meat soaking pad in a Styrofoam tray, over-wrapped with fresh keeping films at 4℃, and displayed for 96 h postmortem with white light of 3500 K [210].

In order to investigate effects of maternal VD_3 on meat quality technological properties in offspring pigs during frozen storage. *Longissimus dorsi* muscle samples of offspring pigs were frozen at -20℃ until the geometric center temperature reached approximately -18℃. After then, all samples of *longissimus dorsi* muscles were stored at -18℃ for 0, 24, 48, 72 and 96 h, respectively. 6 chops for every group (at each storage time) were used for analysis.

In order to investigate effects of maternal VD_3 status on quality and technological properties of pork batters in offspring pigs during cold storage. After

slaughtering, the carcass was split longitudinally. The *longissimus dorsi* muscle was divided into three parts and refrigerated at 4℃ for 0, 24 and 48 h, respectively. Muscle sample was grinded using a meat grinder (6 mm), and weighed 200 g, added 20% ice water and 2% NaCl to each sample for analysis.

2.3 Research Methods

2.3.1 Real-time PCR

Table 2-4 Primer sequences used for RT-PCR

Gene	Accession no.	Primer sequence	Size (bp)
VDR	NM_001097414.1	F: 5'-CGGCAGCCAGCACTTCCTTAC-3'	211
		R: 5'-CGGCGGTTGTCCTTGGTGATG-3'	
PPARγ	NM_214379	F:5'-GACTCAGCTGTACAACAAACCTC-3'	185
		R: 5'-GACAGTTAAGATCGCACCTATC-3'	
FABP₄	NM_001002817	F: 5'-GAAAGAAGTGGGAGTGGGCTTT-3'	212
		R: 5'-GGGCGCCTCCATCTAAGGTTAT-3'	
ZFP423	NC_010448.4	F: 5'-CACCTGACCGTGCACTACAT-3'	128
		R: 5'-CAGTGGTACAGCACGAAGGT-3'	
HSL	AF141958.1	F: 5'-CTGGCGGAGGACAACATGGC-3'	268
		R: 5'-AGAAGATGCTGCGGCGGTTG-3'	
FAS	AY952929	F: 5'-CTACGAGGCCATTGTGGACG-3'	148
		R: 5'-AGCCTATCATGCTGTAGCCC-3'	
RXRα	DQ279926.1	F: 5'-ACGAGGACATGCCGGTGGAG-3'	273
		R: 5'-TGGAGCGGTGCGAGAAGGAG-3'	
ACTB	XM_021086047	F: 5'-ACCTTCTACAACGAGCTGCGTG-3'	207
		R: 5'-GTCTCCGGAGTCCATCACGATG-3'	

VDR, Vitamin D receptor. *PPAR*γ, peroxisome proliferator-activated receptor-γ. *FABP₄*, fatty acid binding protein 4. *ZFP423*, zinc finger protein 423. *HSL,* hormone-sensitive lipase. *FAS,* fatty acid synthase. *RXRα,* retinoid X receptor alpha. *ACTB*, beta-actin.

At 28 and 150 days of age, total RNA of *longissimus dorsal* muscle was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and then removed DNA via DNase treatment (NEB, Ipswich, MA, USA). Approximately 1 μg of the total RNA in each sample was used to synthesize cDNA by PrimeScript™ RT Reagent Kit (Takara Bio Inc., Tokyo, Japan). RT-PCR was performed with ViiA™ 7 real-time PCR System (Applied BioSystems, Foster City, CA, USA) using a SYBR green RT-PCR kit from Bio-Rad (Hercules, CA, USA). Primer sequences were designed according to the basis of known sequences deposited in GenBank (Table 2-4). Relative expression of *ZFP423, VDR, PPARγ, RXRα, HSL, FAS,* and *FABP4* mRNA was determined after normalization to *β*-actin reference using $2^{-\triangle\triangle Ct}$ method.

2.3.2 Serum biochemistry analysis

At 28 days of age, serum leptin levels were measured with a commercially available kit (Multispecies Radioimmunoassay Kit; Linco Research, St. Charles, MO). Insulin, insulin-like growth factor I (IGF-I), free triiodothyronine (FT_3) and free thyroxine (FT_4) concentrations were measured with the RIA kits (Beijing North Institute of Biotechnology, Beijing, China) in a Gamma-counter (Packard 8500, Packard Instrument Co., Downers Grove, Illinois, USA). Serum 25OHD concentration was determined using an EIA kit (IDS Immunodiagnostic Systems Ltd., Tyne and Wear, UK) according to pervious method described by previous study [211].

At 150 days of age, Serum 25OHD concentration was determined using an EIA kit (IDS Immunodiagnostic Systems Ltd., Tyne and Wear, UK) according to the method described by previous researcher [211]. Insulin concentrations were measured with the RIA kits (Beijing North Institute of Biotechnology, Beijing, China) in a Gamma-counter (Packard 8500, Packard Instrument Co., Downers Grove, Illinois, USA). Leptin levels were measured with a commercially available kit (Multispecies Radioimmunoassay Kit; Linco Research, St. Charles, MO). Serum FFA and TG concentrations were determined with enzymatic colorimetric procedure (Nanjing Jiancheng Bioengineering Institute, China) in a UV-visible spectrophotometer (Ultrospec 2000, Sweeden).

2.3.3 Fatty Acids composition analysis

At 150 days of age, for fatty acid analysis, was extracted from *longissimus dorsi* muscle sample according to the methods based on fatty acid methyl ester (FAME) synthesis previous reported [212-214]. Briefly, 0.5 g of freeze-dried sample, 0.7 mL of KOH, and 5.3 mL of methanol were placed in a Pyrex screw cap tube and mixed. The tube was incubated at 50℃ in water for 1.5 h. After cooling in the cold-water bath, 0.58 mL of H_2SO_4 was added in the tube. The tube was mixed and incubated in 50℃ in water for 1.5 h again. It was cooled, 3 mL of hexane were added, and the mixture was mixed for 5 min after FAME synthesis. The fatty acid composition was analyzed on an Agilent Technologies 7890 A gas chromatograph (GC; Model 7890A, Agilent Technologies, Palo Alto, CA) equipped with a DB-23 60 m×0.25 mm capillary column with a 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA). The operating program was as follows: infection volume of 1 μL, injector temperature of 250℃, detector temperature of 300℃, and initial column temperature of 140℃ for 5 min that was then raised to 220℃ at 5℃ per min and kept for 16 min. The fatty acids were determined by a flame ionization detector, chromatograms were analyzed with the use of a gas chromatography chemstation software (Agilent Technologies, Palo Alto, CA). Results of fatty acids were expressed as the percentage of the total fatty acids identified and grouped as follows: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). In addition, the PUFA: SFA and n-6: n-3 ratio was also calculated.

2.3.4 pH value measurement

pH values of *longissimus dorsi* muscle in offspring pigs were determined using a pH meter (Model PC 510, Cyber scan, Singapore) at 24, 48 and 96 h postmortem after slaughter, respectively.

2.3.5 Shear force

Shear force values of *longissimus dorsi* muscle in offspring pigs were carried out with CLM-4 digital explicit muscle tenderness meter (School of Engineering,

Northeast Agricultural University). Briefly, each *longissimus dorsi* muscle was cut into 3 cuboid meat columns along the muscle fibers direction with approximately a width of 1 cm, a height of 1 cm and a length of 2-3 cm. And then, each of cuboid meat columns was sheared along the vertical myofibril direction at 24, 48 and 96 h postmortem after slaughter, respectively. The value of force record during shearing was collected, and the average value was calculated.

In addition, thawed meat samples were also used to determine shear force with CLM-4 digital explicit muscle tenderness meter (School of Engineering, Northeast Agricultural University). Shear force of thawed meat was calculated according to previous report [209].

2.3.6 Color measurement

Color of *longissimus dorsi* muscle in offspring pigs was performed after storage at 4℃ for 24, 48, and 96 h postmortem using a colorimeter (Konica Minolta CR 410, Sensing Inc, Osaka, Japan) equipped with illuminant D65, 10° standard observer and aperture diameter of 8 mm. The frozen meat samples were thawed in refrigerator at 4℃ for 12 h. And then, the color of thawed samples (*longissimus* dorsi muscles) was aslo analyzed by colorimeter (Konica Minolta CR 410, Sensing Inc, Osaka, Japan). In addition, the exudates from the surface of cooked pork batters are wiped off using absorbent paper. After storage in refrigerator at 4℃, the color of pork batters in offspring pigs was determined with a colorimeter (Konica Minolta CR 410, Sensing Inc, Osaka, Japan). The meat color contained lightness (L^*), redness (a^*) and yellowness (b^*) value, respectively [190].

2.3.7 WHC

WHC of *longissimus dorsi* muscle at 24, 48 and 96 h postmortem storage was measured according to the method described by previous researchers [215-217]. Briefly, 1 gram of meat sample was put into a tube which contained filter paper, and centrifuged at 4℃, 1500×g for 15 min. After centrifugation, meat sample was removed, the WHC of meat sample was calculated by the weight of tube with filter paper before and after drying. The tube was dried at 105℃ for 24 h.

2.3.8 LF-NMR

The transverse relaxation times of *longissimus dorsi* muscles in offspring pigs were determined using LF-NMR imaging analyzer (NMI20-040V-I, Suzhou Newmai Analytical instrument Co., Ltd.) according to the methods described by previous researchers [218, 219]. Briefly, meat samples in offspring pigs were cut to cylinders of 1.5 cm diameter and 3 cm long, and each of the cylinders was put into cylindrical tubes of 1.5 cm diameter and 5 cm high. The transverse relaxation time (T_2) of LF-NMR measurements were performed using Carr-Purcell-Meiboom-Gill pulse sequence at 32℃, 200 μs (time between 90°and 180° pulse), and a resonance frequency of 22.4 MHz. A total of 2000 echoes were obtained from 8 scans, and the interval time between scans was 3 s. Each sample was determined 3 times in parallel, and calculated the average value of relaxation times. The thawed meat samples (1.5 cm in diameter and 3 cm length of cylindrical tube) were placed into cylindrical tubes of 1.5cm diameter and 5cm high for analyzation. In addition, LF-NMR imaging analyzer was used to determine the transverse relaxation times of cooked pork batters of offspring pigs according to the previously reported methods[218, 220]Meanwhile, each meat sample was measured three times in parallel, the average value of relaxation time was calculated.

2.3.9 Thawing loss

The thawing loss was calculated based on the percentage weight ratio of *longissimus dorsi* muscle before and after thawing. The equation is as follows:

Thawing loss (%) = $(M_b-M_a)/M_b$

M_b and M_a represent the weight of the meat sample before and after thawing, respectively.

2.3.10 Cooking loss

Cooking loss of thawing meat samples were packed with a plastic bag and kept in 85℃ water for 20 min until the geometric center temperature reached approximately 75℃. Cooking loss was calculated according to the percentage weight ratio of meat before and after cooking. Meanwhile, Samples of pork batters were separately packed

with a plastic bag and kept in 85℃ waters for 20 min. Samples of cooked pork batters were chilled to room temperature for 30 min. Wipe off the surface exudates with absorbent paper. The cooking loss was calculated according to the percentage weigh ratio of pork batters before and after cooking[216].

$$\text{Cooking loss (\%)} = (M_b - M_a)/M_b \quad (2.1)$$

Where M_b and M_a represent the weight of thawed meat sample before and after cooking.

2.3.11 TPA (Texture profile analysis)

The cooked pork batters were stored in refrigerator at 4℃ for 0, 24 and 48 h, and then sorted into cylinders with a diameter of 2 cm and a height of 3 cm. And then, the texture of the samples was determined using P/36R probe. Each sample was measured three times in parallel, and then the average values of springiness, hardness, chewiness, and cohesiveness were analyzed.

2.3.12 Preparation of pork batters

The technological process for preparing pork batters is as follows: Pork (4 degree cold storage)→mincing→chopping and mixing (low temperature) →centrifugation (removing bubbles)→raw pork batters→cooking (forming gel)→cooked pork batters (measuring color difference, cooking yield and texture).

Take out the pork after refrigeration, use a meat grinder (6mm) to grind it and weigh 200 g, add 20% ice water and 2% NaCl to each sample. The specific methods are as follows:

Put pork and NaCl into the chopper, chop and mix at 1500 r/min for 30 s, and slowly add 1/3 ice water; then chop and mix at 1500 r/min for 30 s, and slowly add 1/3 ice water again; Finally, chop and mix for 60 s at 3000 r/min, and slowly add the remaining 1/3 of ice water (the center temperature is lower than 10℃). Take 35 g of pork batters and put it into a 50 mL centrifuge tube. Centrifuge the pork batters for 3 min at 500 r/min to completely remove the bubbles in the pork batters, and then cook it in a water bath at 80℃ for 25 min (the center temperature is 72 ℃). Cool it in ice water mixture to about 20℃, and put it into a refrigerator at 4℃ for overnight.

2.4 Statistical analysis

Statistical analysis of variance (ANOVA) was performed using the one-way ANOVA procedure of SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The post-hoc analysis for comparing group means (offspring pigs) were measured by Duncan's multiple range tests, and significance was declared at $P<0.05$. Genes expression analysis was performed using REST 2009 software (https://www.gene-quantification.de/rest-2009.html). Replicate was used as experimental materials unit for the study of carcass traits, meat quality, serum biochemical indicators and gene expression. Bivariate correlations were used to evaluate the correlation between meat quality (carcass fat, IMF content and ABFT) and adipogenic gene (*FAS* and *HSL*) expression.

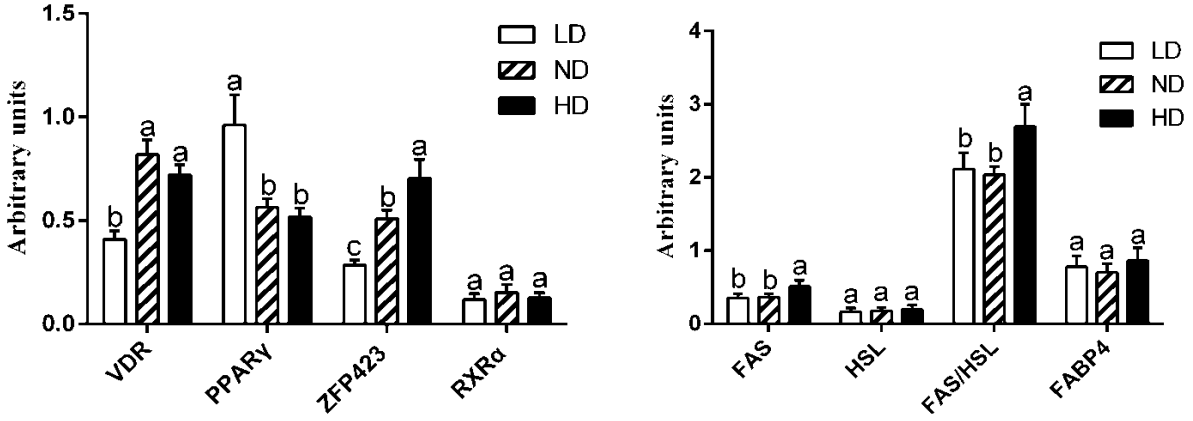
2.5 Conclusions in section 2

1. Methodological approaches adopted in the dissertation work that include theoretical research, feeding experiment, slaughter experiment, meat quality test analysis, and its subordinate research methods, which solved the scientific problems of this dissertation.
2. The object of research of dissertation work is determined- Meat production technology with given functional and technological properties, the level of VD_3 in sows during pregnancy, lipid metabolism and the quality of offspring meat.
3. Research items- Pork meat, functional and technological properties of meat, differential maternal status of VD_3 sow, offspring.
4. The selected set of methods allows you to comprehensively characterize the formation of functional and technological properties of pork by the influence of Vitamin D_3 on the differential maternal level of pigs.

SECTION 3 RESULTS OF THE STUDY OF THE EFFECT OF VD3 ON THE POTENTIAL FOOD FACTOR OF PORK

3.1 Effects of maternal VD3 on factors related to the formation of meat quality function in offspring piglets

3.1.1 Gene expression



3-**1A** **3-1B**

Figure 3-1A. *VDR*, *PPARγ*, *ZFP423* and *RXRα*mRNA expression in musculus *longissimus dorsi* of piglets born to LD, ND and HD groups. **Figure 3-1B.** *FAS, HSL, FAS/HSL* and *FABP4* mRNA expression in musculus *longissimus dorsi* of offspring born to LD, ND and HD groups.

As shown in Figure 3-1A, *PPARγ* expression in *longissimus dorsi* muscle of piglets born to LD group was significantly higher than those born to the ND and HD groups, respectively ($P<0.05$). Whereas, *VDR* expression of piglets born to LD group was lower than those born to the ND and HD groups, respectively ($P<0.05$). No differences in *PPARγ* and *VDR* expression of piglets between ND and HD groups ($P>0.05$). In addition, *ZFP423* expression in piglets born to ND group was higher than those born to the LD groups, while lower than those born to the HD group, respectively ($P<0.05$). No differences in *RXRα* expression of piglets among all groups (LD, ND and HD groups, $P>0.05$).

As shown in Figure 3-1B, there were no differences in *HSL* and *FABP4* expression of offspring piglets among all groups ($P>0.05$). Whereas, *FAS* expression and *FAS/HSL* ratio in piglets born to HD group was higher than those born to LD and

ND groups (P<0.05), and no differences were observed between LD and ND groups (P>0.05).

3.1.2 Blood biochemical index

Table 3-1. Effects of maternal VD_3 on serum hormonal and biochemical index in offspring piglets

Item	Groups			S.E.M	P-value
	LD	ND	HD		
Leptin (ng/mL)	0.56^{c}	0.74^{b}	0.95^{a}	0.080	0.001
IGF-I (ng/mL)	41.79^{a}	34.51^{b}	24.89^{c}	0.975	0.001
FT3 (fmol/mL)	0.74^{a}	0.56^{b}	0.45^{b}	0.070	0.004
FT4 (fmol/mL)	5.03^{a}	4.26^{b}	3.60^{b}	0.427	0.015
25 (OH)D (nmol/mL)	13.44^{c}	15.52^{b}	17.13^{a}	0.569	0.015
Insulin (μmol/mL)	6.63^{b}	7.88^{a}	8.19^{a}	0.574	0.007

In the same line, values with different small letter superscripts mean significant difference (P<0.05).

As shown in Table 3-1, serum leptin and 25(OH)D concentrations in piglets born to ND group were higher than those born to LD groups, and lower than those born to HD groups, respectively (P<0.05). Serum IGF-I concentration of ND group was lower than that of LD group, and higher than that of HD group, respectively (P<0.05). In addition, serum FT3 and FT4 levels in LD groups were higher than those in ND and HD groups, while insulin concentration was lower than those in ND and HD groups, respectively (P<0.05). No differences were observed in FT3, FT4 and insulin concentrations between ND and HD groups (P>0.05).

3.1.3 Discussion

This investigation aimed to explore the impact of maternal VD_3 status (excess or deficiency) during pregnancy on adipogenesis genes expression and serum biochemical index in offspring piglets. We found that there were differences in expression of *PPARγ, VDR*, *ZFP423, FAS, FAS/HSL* ratio, and serum IGF-I, FT3, FT4, leptin, 25(OH)D, insulin concentration in piglets. These results suggested that maternal

VD_3 during pregnancy have lasting effects on factors regulating adipogenesis in their piglets. Adipogenesis process is mediated by many transcription factors, such as *ZFP234, PPARγ, C/EBPα, FABP*$_4$, *SCD-1*and so on [179]. Previous research shown that VD_3 is recognized as a potential regulator of adipogenesis [36], and it inhibited differentiation and adipogenesis of 3T3-L1 preadipocytes by decreasing *PPARγ*, *C/EBPα, FABP*$_4$ and *SCD-1* expression [102]. In vitro studies also shown that VD inhibited porcine preadipocyte differentiation by decreasing *PPARγ* and *RXRα* expression [103]. *PPARγ* is the critical regulator of adipogenesis, which can stimulate adipogenesis [62]. In this present study, maternal VD_3 deficiency (LD group) increased *PPARγ* expression of *longissimus dorsi* muscle in offspring. These results indicated that maternal VD_3 status can influence adipogenesis in *longissimus dorsi* muscle of piglets by alternating *PPARγ* mRNA expression. Similar results were observed by previous researcher [62], who found that VD deficient-exposed offspring mice had greater *PPARγ* expression in perigonadal white adipose tissue.

VDR has an inhibitory effect on adipogenesis. Previous results shown that *VDR* expression in 3T3-L1 cells inhibited *PPARγ* mRNA levels and decreased adipogenesis, which suggested that *VDR* inhibited adipogenesis via decreasing *PPARγ* expression [100, 102]. In present study, maternal VD_3 deficiency decreased *VDR* mRNA expression in piglets. These results indicated that maternal VD_3 deficiency increased *PPARγ* by decreasing *VDR* expression, and further enhanced adipogenesis [100]. The molecular mechanism of inhibitory effect of *VDR* on adipogenesis maybe due to *RXR* is a heterodimeric partner for both *PPARγ* and *VDR* respectively, and that *VDR* competes *RXR* with *PPARγ* to decrease adipogenesis [36, 102]. Inconsistent results were reported by previous researcher [62], who observed that VD deficient-exposed offspring mice had higher *VDR* expression. These differences may be due to different species, tissue, as well as duration of feeding VD_3.

ZFP423 is identified as a key transcriptional initiator of adipogenic differentiation in adipose tissue, which promoting adipogenic commitment and adipogenesis [31]. Previous research also found that vitamin A administration at birth could increase *ZFP423* expression, and promote intramuscular fat development in

Angus beef cattle [221]. In addition, maternal vitamin A administration expanded PDGFRa$^+$ adipose progenitor population in progeny, and the PDGFRa$^+$ progenitors can further differentiate into white adipocytes, and promote adipogenesis [222, 223]. In current study, maternal VD_3 supplementation increased the *ZFP423* expression in *longissimus dorsal* muscle of piglets. These results indicated that maternal nutrition could affect fetal epigenome by improving the intrauterine environment [140, 224], and maternal VD_3 could promote adipogenic commitment of progenitor cells in *longissimus dorsi* muscle by increasing *ZFP432* expression of offspring piglets. The molecular mechanism might be due to maternal VD_3 increased *ZFP423* expression which increasing PDGFa$^+$ progenitor population in *longissimus dorsi* muscle of piglets, and further promoting the numbers of intramuscular adipocytes.

Meanwhile, adipose tissue deposition depends on the balance between lipid synthesis and degradation [51, 52]. This process is regulated by *FAS* and *HSL* expression. In this study, *FAS* expression and *FAS/HSL* ratio in piglets born to HD group was higher than those born to the ND and LD groups. Similar results were reported that VD_3 promoted adipocyte differentiation in 3T3-L1 cells and induced *FAS* expression [225, 226]. Other research observed that VD_3 stimulated *FAS* expression in primary human adipocytes and adipose organ cultures [227, 228]. These data suggested that maternal VD_3 could increase lipid accumulation in *longissimus dorsi* muscle of piglets by enhancing *FAS* expression and *FAS/HSL* ratio. The one of possible reason may be that maternal VD_3 increased adipogenic gene expression by enhancing PDGFRa$^+$ adipose progenitor population [222, 223]. Certainly, the molecular regulatory mechanism underlying still needs to be proved by further investigation.

Leptin is an important regulator of energy homeostasis in mammals, and adipocytes can produce and secrete leptin [57]. Circulating leptin levels are strongly correlated with adipose tissue stores [58]. In this study, serum leptin concentration in piglets born to HD groups was higher than those born to ND and LD groups. The data suggested that high levels of maternal VD_3 supplementation probably increase the adipose accumulation in *longissimus dorsi* muscle, and then further enhance serum leptin levels of offspring. In mice, maternal VD deficiency did not affect serum leptin

in offspring mice [62]. Inconsistent results in serum leptin concentrations may be due to differential species and dosage of feeding VD_3. Previous results shown that IGF-I, T_3 and T_4 can increase basal energy expenditure through altering lipid metabolism pathway [56]. In this study, it was observed that serum IGF-I, FT_3 and FT_4 levels in offspring piglets born to LD group were higher than those born to ND and HD groups, which indicated that maternal VD_3 deficiency increased basal energy expenditure and lipolysis in *longissimus dorsi* muscle of piglets. Insulin is able to increase glycogen synthesis and fat deposition, while decrease gluconeogenesis. In present study, serum insulin levels of offspring piglets born to LD group were lower than those born to ND and HD groups, which indicated that maternal VD_3 deficiency decreased lipid synthesis in *longissimus dorsi* muscle of piglets via decreasing serum insulin levels. Other research found that no differences in serum insulin levels between offspring mice born to maternal VD deficiency and those born to the control groups [62]. Inconsistent research results in serum insulin might be due to differential species, tissue and dosage of VD, but the reasons and its mechanism have not been unclear.

Serum 25(OH)D concentration usually reflects the VD status of pigs [229]. In this present study, serum 25(OH)D concentration in piglets born to LD group was lower than those of ND and HD groups, which indicated that maternal VD_3 deficiency decreased the serum 25(OH)D concentration in early piglets (at 28 days of life). Similar result was reported by previous researcher [61], who observed that maternal dietary VD influenced serum 25(OH)D_3 levels in piglets (early after weaning). Other reports shown that enhancing maternal VD_3 supplementation increased serum 25(OH)D_3 in offspring [230]. Maternal VD deficiency groups decreased serum 25(OH)D concentration of offspring mice [117]. Whereas, there were no differences in serum 25(OH)D_3 levels of offspring mice between born to VD deficient and normal groups. Inconsistent results may be due to different species. Taken together, maternal VD_3 could also affect adipogenesis in *longissimus dorsi* muscle of piglets by altering serum biochemical parameters. Certainly, its mechanism underlying still needs to be identified by further investigation.

3.2 Effects of maternal VD_3 on the formation of meat quality function and its regulatory factors in offspring pigs

3.2.1 Carcass characteristics and meat quality

Table 3-**2** Carcass characteristics and meat quality in offspring pigs

Item	Treatment			S.E.M.	*P*-value
	LD	ND	HD		
Live weight, kg	94.89	92.72	94.27	0.95	0.224
Carcass weight (kg)	66.44	67.05	68.45	0.568	0.440
Dressing percentage (%)	70.02	72.67	72.61	0.589	0.910
Carcass fat (g/100g)	26.45	23.21	22.18	0.227	0.001
ABFT (cm)	1.96	1.41	1.34	0.052	0.001
Marbling score	1.47b	1.87a	1.93a	0.07	0.001
Subjective color score	2.71b	2.83b	3.19a	0.10	0.004
Longissimus muscle area (cm^2)	32.42c	33.60b	35.39a	0.43	0.002
Drip loss (%)	4.82a	4.61a	4.18b	0.15	0.002
Shear force (Newton)	35.28a	31.36b	27.44c	1.33	0.002
IMF (g/100g)	1.62	1.83	1.87	0.021	0.001

ABFT, average backfat thickness. IMF, intramuscular fat. SEM, Standard error of the mean.

The effects of maternal VD_3 during pregnancy on carcass characteristics and meat quality in offspring pigs are shown in Table 3-2. There were no significant differences in carcass weight (*P*=0.440) and dressing percentage (*P*=0.910) among all groups. The offspring pigs born to LD group had higher carcass fat content ((*P*=0.001) and ABFT (*P*=0.001) compared with the ND and HD groups, respectively. Whereas, IMF content of *longissimus dorsal* muscle in offspring pigs born to LD group was lower than those born to the ND and HD groups, respectively (*P*=0.001). In addition, no significant differences in carcass fat, ABFT and IMF content were measured between the ND and HD groups.

Meanwhile, offspring pigs from the LD group pigs had lower marbling score in comparison with those from ND and HD groups (P<0.05), while they had higher drip loss in comparison with those from HD group (P<0.05). meanwhile, the subjective color score in offspring pigs born in HD group was higher than those born in LD and ND groups (P<0.05). Additionally, *longissimus dorsi* muscle area of offspring pigs from to ND groups was lower than that in pigs from HD group but higher than that in pigs from LD group (P<0.05), whereas, the shear force of the *longissimus dorsi* muscle of the ND group was lower than that of the LD group and higher than that of HD group (P<0.05). There was no difference in the average live weight among all groups (P>0.05).

3.2.2 Fatty acids composition of pork in offspring

Table 3-3 Effects of maternal VD_3 on fatty acids composition of *longissimus dorsi* muscle in offspring pigs

Item	Groups			SEM	*P*-value
	LD	ND	HD		
SFA[1]					
C14:0 (%)	2.58[a]	2.22[b]	2.13[b]	0.14	0.002
C16:0 (%)	27.68[a]	25.22[b]	24.21[b]	0.32	0.001
C18:0 (%)	13.24[a]	11.69[b]	10.35[b]	0.12	0.002
C20:0 (%)	0.16[a]	0.15[a]	0.14[a]	0.09	0.062
MUFA[2]					
C16:1 (%)	1.88[b]	2.24[a]	2.46[a]	0.04	0.001
C18:1n-9 (%)	32.55[b]	33.21[a]	34.44[a]	0.34	0.044
C20:1 (%)	1.07[b]	1.14[a]	1.16[a]	0.03	0.031
PUFA[3]					
C18:2 n-6(%)	18.84[b]	21.43[a]	21.26[a]	0.09	0.001
C18:3 n-3(%)	0.63[b]	0.84[ab]	1.06[a]	0.04	0.039
C20:2 n-6(%)	0.31[b]	0.38[ab]	0.46[a]	0.02	0.042
C20:3 n-3(%)	0.81[b]	0.94[ab]	1.14[a]	0.06	0.046

Continuation of Table 3-3

Item	Groups			SEM	*P*-value
	LD	ND	HD		
C20:4 n-6(%)	0.25[b]	0.34[a]	0.39[a]	0.02	0.021
Total SFA (%)	43.66[a]	39.28[ab]	36.83[b]	0.62	0.014
Total MUFA (%)	35.50[b]	36.59[ab]	38.06[a]	0.26	0.003
Total PUFA (%)	20.84[b]	24.13[a]	25.11[a]	0.19	0.001
Total PUFA:SFA	0.48[b]	0.61[a]	0.68[a]	0.02	0.002
Total n-6:n-3	13.47[a]	12.55[ab]	10.41[b]	0.16	0.001

In the same column, values with different small letter superscripts mean significant difference ($P<0.05$). [1] saturated fatty acid, [2] monounsaturated fatty acid, [3] polyunsaturated fatty acid.

As shown in Table 5-2, the offspring pigs born to LD group had higher total SFA concentrations compared with those born HD groups ($P<0.05$), and no differences were observed between LD and ND, as well as HD and ND groups respectively ($P>0.05$). The C14:0, C16:0, and C18:0 concentrations of offspring pigs born to ND and HD groups were significantly lower than that born to LD group ($P<0.05$), and no differences were observed between ND and HD groups ($P>0.05$). In addition, there were no differences in C20:0 concentrations among all groups ($P>0.05$).

Total MUFA concentrations of *longissimus dorsi* muscle in offspring pigs born to HD group were higher than that born to LD group ($P<0.05$), and no differences were observed between LD and ND, as well as HD and ND groups respectively ($P>0.05$). Meanwhile the offspring pigs born to HD and ND groups had higher the concentrations of C16:1, C18:1 and C20:1 compared with those born to LD group ($P<0.05$).

The offspring pigs born to ND and HD groups had higher total PUFA concentrations and total PUFA: SFA ratio compared with offspring pigs born to LD group ($P<0.05$). In addition, the levels of C18:2 and C20:4 of offspring pigs born to ND and HD groups were significantly higher than those born to LD group ($P<0.05$). The C18:3, C20:2 and C20:3 levels in offspring pigs born to HD group were higher, while total n-6:n-3 ratio were lower than those born to LD group ($P<0.05$), no

differences were determined between LD and ND, as well as ND and HD group, respectively (P>0.05).

3.2.3 Serum biochemical index

As shown in Table 3-4, no significant differences in serum 25OHD concentration were observed among all groups (P=0.376). The offspring pigs born to LD group had higher concentrations of serum insulin (P=0.001) and leptin (P=0.010) compared with the ND and HD groups, respectively. Whereas, serum FFA (P=0.020) and TG (P=0.026) concentrations of offspring pigs born to the LD group were lower than those born to the ND and HD groups, respectively. Meanwhile, the offspring pigs born to the HD group had lower serum insulin, leptin, and higher FFA, TG levels compared with the ND group, respectively.

Table 3-4 Serum biochemical index in offspring pigs

Item	Treatment			S.E.M.	P-value
	LD	ND	HD		
25OHD (ng/mL)	9.68	9.85	9.97	0.343	0.376
Insulin (μmol/mL)	16.61	12.48	10.15	0.810	0.001
Leptin (ng/mL)	1.62	1.34	1.13	0.072	0.010
FFA (μmol/L)	352.60	420.44	443.39	6.018	0.020
TG (mmol/L)	1.09	1.34	1.53	0.066	0.026

FFA, free fatty acids. TG, triacylglycerol.

3.2.4 *FAS* and *HSL* gene expression

As shown in Table 3-5, *FAS* mRNA expression (P=0.009) and the ratio of *FAS/HSL* mRNA expression (P=0.002) in subcutaneous adipose tissue of offspring pigs born to LD group was higher than those born to the ND and HD groups, respectively. Meanwhile, offspring pigs born to HD group had lower expression of *FAS* and the ratio

of *FAS/HSL* mRNA expression compared with the ND group. Whereas, no differences in *HSL* expression were observed among all groups (*P*=0.268).

Table 3-5 *FAS*, *HSL* and the ratio of *FAS/HSL* mRNA expression in subcutaneous adipose tissue of offspring pigs at 150 days of age.

Gene	Treatment			S.E.M.	*P*-value
	LD	ND	HD		
FAS	1.242	0.955	0.638	0.128	0.009
HSL	1.194	1.277	1.352	0.087	0.268
FAS/HSL	1.044	0.745	0.475	0.088	0.002

Table 3-6 *FAS*, *HSL* and the ratio of *FAS/HSL* mRNA expression in *longissimus* dorsal muscle tissue of offspring pigs at 150 days of age.

Gene	Treatment			S.E.M.	*P*-value
	LD	ND	HD		
FAS	1.908	1.348	1.863	0.119	0.006
HSL	2.163	1.187	1.290	0.068	0.001
FAS/HSL	0.884	1.132	1.447	0.088	0.011

SEM, Standard error of the mean.

As shown in Table 3-6, offspring pigs born to LD group had lower the ratio of *FAS/HSL* mRNA expression in *longissimus dorsal* muscle compared with those born to the ND and HD groups, respectively (*P*=0.011), and the ratio of *FAS/HSL* mRNA expression in offspring pigs born to ND group was lower than that born to the HD group (*P*=0.011). Compared with the LD group, the ND and HD groups had lower expression of *HSL* mRNA in *longissimus dorsal* muscle (*P*=0.001). The *FAS* mRNA expression in the ND group was lower than that in the LD and HD groups, respectively (*P*=0.006). Whereas, no differences in *FAS* mRNA expression between the LD and HD groups, as well as *HSL* expression between the ND and HD groups, respectively.

3.2.5 Correlation between genes expression and meat quality parameters

Table 3-7 Correlations between *FAS* and *HSL* mRNA expression levels and carcass fat content, ABFT and IMF in offspring pigs

Item	*FAS* mRNA levels	*HSL* mRNA levels	*FAS/HSL*
Longissimus dorsal muscle tissue			
IMF	r=-0.409, *P*=0.275	r=-0.561, *P*=0.116	r=-0.868*, *P*=0.002
Subcutaneous adipose tissue			
Carcass fat content	r=0.843*, *P*=0.004	r=-0.555, *P*=0.121	r=0.890**, *P*=0.001
ABFT	r=-0.746*, *P*=0.021	r=-0.475, *P*=0.196	r=0.795*, *P*=0.010

As shown in Table 3-7, the ratio of *FAS/HSL* mRNA expression in *longissimus dorsal* muscle was negatively correlated to IMF content of offspring pigs (r=-0.868, *P*=0.002). Whereas, there is no relation between *FAS*, *HSL* mRNA expression and IMF content. In addition, the *FAS* mRNA expression (r=0.843, *P*=0.021) and the ratio of *FAS/HSL* mRNA expression (r=0.890, *P*=0.001) in subcutaneous adipose tissue were both positively correlated with carcass fat content. Meanwhile, the *FAS* mRNA expression was negatively correlated to the ABFT (r=-0.746, *P*=0.021). Whereas, the ratio of *FAS/HSL* mRNA expression was positively correlated to ABFT in offspring pigs (r=0.795, *P*=0.010).

3.2.6 Discussion

Previous research showed that production efficiency and meat quality in mammals (including cattle, sheep and pigs) were affected by nutrient fluctuations during the fetal stage [7]. Yang et al. (2013) also found that offspring growth performance was impacted by nutrition concentrations during gestation [24] . Our research observed that there were differences in carcass fat content, ABFT, and IMF content in offspring pigs born to LD group compared with the ND group. These results suggested that maternal VD_3 status could improve growth performance, carcass characteristics and meat quality of offspring pigs. These results are in accordance with previous researches.

Belenchia et al. (2018) observed that maternal VD concentrations during pregnancy have lasting effects on adipose tissue development in offspring mice [62]. Zhou et al. (2016) also found that improving maternal VD status promoted postnatal skeletal muscle development of pig offspring [174]. Flohr et al. (2016) observed that pigs from sows fed 50 μg/kg 25(OH)D_3 had higher ADG (average daily gain) compared with pigs from sows fed 800 of VD_3, and higher final body weight and hot carcass weight compared with pigs from sows fed 9600 IU of VD_3 [61]. These data indicated that maternal VD supplementation affected subsequent growth performance and carcass characteristics in offspring pigs. In addition, offspring pigs born to LD group had higher carcass fat and ABFT compared with the ND and HD groups, which suggested that maternal VD_3 deficiency could increase adipogenesis in adipose tissue of offspring pigs. The reason may be supported that maternal VD_3 inhibited the differentiation of preadipocyte, decreased the number of preadipocytes in fetal, which reduced the fat deposition in offspring pigs. Similar results are reported by previous researcher [100], who demonstrated that VD_3 decreased 3T3-L1 preadipocyte differentiation by inhibiting adipogenic genes expression. Wang et al. (2017) observed that maternal vitamin A administration expanded $PDGFRa^+$ adipose progenitor population in offspring mice[222]. $PDGFRa^+$ adipose progenitor is differentiated into both beige and white adipocytes [223]. In this study, we observed that maternal VD_3 increased IMF content in offspring pigs. The reason may be that maternal VD_3 increased $PDGFRa^+$ adipose progenitor population which differentiated into white adipocytes in *longissimus dorsal* muscle, thereby enhanced IMF content in offspring pigs. Certainly, the mechanism underlying still needs to be proved by further investigation.

In the presented study, the marbling score and the subjective color score of offspring pigs were influenced by maternal VD_3 levels. Both marbling score and subjective color score in offspring from HD group were higher than that from ND and LD groups. So it was revealed that maternal VD_3 supplementation improved meat quality in offspring. The reason may be that the high dosage of maternal VD_3 increase the IMF content of offspring pigs. In the present study, the offspring from HD group showed better meat quality since they were lower pale, drip loss, shear force, and higher

longissimus dorsi muscle area compared with ND and LD groups. These results suggested that meat quality of offspring pigs could be influenced by maternal VD_3 status during pregnancy. Such results may suggest that meat quality in offspring pigs can be influenced by maternal VD_3 status during pregnancy. Similar results were also found by our previous study [231], in which found that maternal VD_3 supplementation improved meat quality of offspring pigs during frozen storage by inhibiting the decrease of cooking loss, drip loss and thawing loss of *longissimus dorsi* muscle. The previous and present results indicated that high-dose maternal VD_3 supplementation can prolong the storage time of pork quality in offspring during cold and frozen storage, respectively.

Growing evidence has shown that meat quality is associated with its IMF content and fatty acid composition in animals [232]. The increase in the degree of lipid unsaturation in meat is beneficial for human health [233]. lower SFAs and higher n-3 fatty acids concentrations will improve meat quality of animals, which reduces cardiovascular diseases risk[234]. In this study, maternal VD_3 supplementation during pregnancy decreased total SFAs, and increased n-3 PUFA concentrations in offspring pigs, which indicated that maternal high-dose VD_3 levels regulated fatty acids composition of *longissimus dorsi* muscle in offspring. Meanwhile, the higher SFAs and ratio of n-6:n-3 PUFA is associated with the pathogenesis of many diseases, such as diabetes, coronary heart disease, cancer, cerebrovascular and cardiovascular diseases of humans[235, 236]. This study has shown that the total SFAs and total n-6:n-3 ratio of *longissimus dorsi* muscle in offspring pigs were reduced by maternal VD_3 supplementation. These results indicated that maternal VD_3 supplementation regulated meat quality by affecting the composition of fatty acids. In conclusion, maternal high-dose VD_3 supplementation during pregnancy can affect the fatty acids composition and meat quality parameters of offspring pigs. But its mechanism is still unclear, which needs to be further explored in future research.

Serum 25OHD is the liver metabolite and primary circulating form of VD, and used to determine the VD status of pigs [229]. In this study, no differences in serum VD status were observed among all groups, which indicated that maternal VD_3 didn't

changed the serum VD concentration in later offspring pigs (at 150 days of age). Similar result was reported by Flohr et al. (2016) [61], who observed that maternal VD influenced serum concentration in growing offspring pigs until 35 days post weaning. Flohr et al. (2014) demonstrated that serum VD_3 of weaned pigs (21 days of age) was also affected by maternal VD status [230] . These results suggested that maternal VD_3 during pregnancy mainly affected the serum VD levels in early offspring pigs, but had no significant effect on the later offspring pigs. Previous study has shown that insulin suppressed lipolysis of rats by increasing *FAS* and acetyl-COA carboxylase (*ACC*) expression, which indicated that lipogenesis is regulated by insulin [237]. Whereas, leptin could induce lipolysis and inhibits lipogenesis [238]. Serum 25OHD decreased leptin concentrations, which was negatively associated with leptin levels [239]. Meanwhile, VD deficiency is correlated with elevated insulin resistance, which regulated lipid metabolism process [240]. In addition, offspring pigs born to LD group had higher serum insulin, leptin levels and lower FFA and TG concentrations compared with the ND and HD groups, which indicated that maternal VD_3 status affected adipogenesis in offspring pigs by regulating the levels of serum biochemical parameters related to lipid metabolism. Similar result was reported by previous researcher [117], who found that maternal VD deficiency increased the adiposity in offspring mice through regulating serum biochemical index concentrations. However, no significant differences in serum insulin and leptin levels in offspring mice were observed between maternal VD deficiency and the control groups [62]. Inconsistent research results in serum biochemical parameters might be due to differential species of animals, dosage of VD, duration of feeding, and feeding methods, but the reasons and its mechanism have not been unclear.

The FAS promotes conversion of acetyl-CoA and malonyl-CoA to TG, which controlling *de novo* lipogenesis of mammals [241]. Whereas, *HSL* mainly catalyzes hydrolysis of stored TG in adipose tissue into FFA and glycerol to regulate lipolysis in animals [54]. Fat accumulation is determined by the balance between *FAS* and *HSL* (the ratio of *FAS/HSL*) expression, *FAS* mRNA expression and the ratio of *FAS/HSL* mRNA expression are positively correlated to carcass fat content in pigs [51]. In this study, we observed that maternal VD_3 deficiency increased carcass fat content and ABFT by

increasing *FAS* mRNA expression and the ratio of *FAS/HSL* mRNA expression. These results are in accordance with previous reports, Yao et al. (2015) demonstrated that obesity is associated with VD deficiency, and VD suppresses adipogenesis [242]. Whereas, Bhat et al. (2014) observed that VD-deficient rats decreased visceral fat content and *FAS* and *PPARγ* expression [118]. Inconsistent research results may be due to differential adipose tissue and species of animals. Meanwhile, our research also found that maternal VD_3 deficiency had lower the ratio of *FAS/HSL* expression in *longissimus dorsal* muscle, and these results are in accordance with lower IMF content. The reason may be that maternal VD_3 deficiency suppressed $PDGFRa^+$ adipose progenitor population which differentiation into white adipocyte in *longissimus dorsal* muscle, further decreased IMF of offspring pigs by decreasing ratio of *FAS/HSL* mRNA expression. So, the ratio of *FAS/HSL* mRNA expression was opposite between the *longissimus dorsal* muscle tissue and subcutaneous adipose tissue with the same VD_3 diet in this study. Certainly, the mechanism underlying still needs to be proved by further investigation.

Taken together, these results indicated that maternal VD_3 status could change adipose tissue metabolism, carcass characteristics and meat quality in offspring pigs by regulating gene expression involved in lipid accumulation. Whereas, the mechanism and signal pathway underlying still needs to be proved by further investigation.

Fat accumulation in adipose tissue is associated with *FAS* mRNA expression [243]. Backfat thickness, a good indicator of fat deposition, is closely correlated with carcass fat and IMF of pigs [244]. In this present experiment, we found that the ratio of *FAS/HSL* mRNA expression in adipose tissue was positively correlated with carcass fat content and ABFT, which suggested that carcass content and ABFT in pigs were affected by the ratio of *FAS/HSL* mRNA expression. Similar results were reported by previous researcher [51], who demonstrated that there was a positive relationship between the ratio of *FAS/HSL* mRNA expression and carcass fat in pigs. Meanwhile, *FAS* mRNA expression was negatively correlated to AFBT, whereas, there was a positive correlation between the ratio of *FAS/HSL* mRNA expression and AFBT. These results confirmed that fat accumulation in subcutaneous adipose tissue of pigs was a balance

between *FAS* and *HSL* expression levels, and fat deposition was increased when *FAS* expression was higher than *HSL* expression. In addition, our study also observed that the ratio of *FAS/HSL* mRNA expression in *longissimus dorsal* muscle was negatively correlated with IMF content in offspring pigs. Similar results were reported by previous researcher [52], who found that there was a negative relationship between the ratio of *FAS/HSL* expression and IMF content in Kazak sheep. However, other study reported that the ratio of *FAS/HSL* expression was positively correlated with IMF content in Sutai pigs [245]. Inconsistent research results may be due to the different patterns of IMF storage or breeds [246]. Taken together, our results in present study indicated that maternal VD_3 status regulated adipogenic genes expression in IMF, whereas, didn't alter relationship between the ratio of *FAS/HSL* expression and carcass fat content, ABFT, as well as IMF content in offspring pigs.

3.3 Conclusions in section 3

1. High-dose VD_3 maternal feeding can improve the pork quality function and technological properties of offspring by regulating key genes and serum hormones related to adipose tissue deposition.
2. Maternal VD_3 status during pregnancy have long-lasting impact on meat quality function and technological properties in offspring pigs.
3. Maternal VD_3 supplementation have positive effects on healthfulness of fatty acid profiles of offspring pork.
4. By adjusting the maternal VD_3 level during pregnancy, the pork quality characteristics of offspring were improved, and the scientific feeding scheme of VD_3 in early pregnancy sows was optimized.

SECTION 4 RESEARCH OF PHYSICAL-CHEMICAL INDICATORS OF MEAT QUALITY AND ITS ABILITY TO BE STORED

4.1 Effects of maternal vitamin D_3 status on pork quality traits in offspring pigs during postmortem storage

4.1.1 pH value

Table 4-1 Effects of maternal VD_3 status on pH value of *longissimus dorsi* muscle in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
24 h	5.74^{Ab}	5.83^{Aab}	5.88^{a}	0.456	0.032
48 h	5.73^{Ab}	5.78^{Aab}	5.83^{a}	0.092	0.044
96 h	5.62^{Bb}	5.65^{Bb}	5.81^{a}	0.136	0.028
SEM	0.322	0.298	0.511	-	-
P-value	0.024	0.038	0.216	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

The effects of maternal VD_3 status on pH value of *longissimus dorsi* muscle in offspring pigs at 24, 48 and 96 h postmortem storage were shown in Table 4-1. Offspring pigs from HD group had higher pH values compared with those from LD group at 24, 48 and 96 h postmortem storage, respectively ($P<0.05$). Meanwhile, the changes of pH value in offspring pigs from all groups followed the same time-dependent patterns, which decreased with postmortem storage time. Offspring pigs from LD and ND groups had higher pH value at 24, 48 h compared with those at 96 h postmortem storage ($P<0.05$). Whereas, no differences in pH value of offspring pigs from HD group were obtained among all postmortem storage times (24, 48, and 96 h, $P>0.05$).

4.1.2 Shear force

Table 4-2 Effects of maternal VD_3 status on shear force of *longissimus* dorsi muscle in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
24 h	3.59^{a}	3.20^{b}	2.80^{Ac}	0.338	0.003
48 h	3.51^{a}	3.11^{b}	2.65^{ABc}	0.324	0.001
96 h	3.42^{a}	3.04^{b}	2.53^{Bc}	0.316	0.009
SEM	0.413	0.311	0.116	-	-
P-value	0.089	0.077	0.024	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

As shown in Table 4-2, shear force of *longissimus dorsi* muscle in offspring pigs from ND group was higher than that from HD group, while was lower than that from LD group at 24, 48, and 96 h postmortem storage, respectively ($P<0.05$). In addition, offspring pigs from HD group had higher shear force at 24 h compared with that at 96 h postmortem storage ($P<0.05$). No differences in shear force of offspring pigs from LD and ND groups were observed among all postmortem storage times ($P>0.05$).

4.1.3 WHC

As shown in Table 4-3, WHC of *longissimus dorsi* muscle in offspring pigs from HD group was higher than those from LD group at 24, 48, and 96 h postmortem storage, respectively ($P<0.05$). And offspring pigs born to LD group had higher WHC of *longissimus dorsi* muscle at 24 h compared with that at 96 h postmortem storage ($P<0.05$). However, no differences in WHC of offspring pigs from ND and HD groups was obtained among all postmortem storage times ($P>0.05$).

Table 4-3 Effects of maternal VD_3 status on WHC of *longissimus dorsi* muscle in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
24 h	75.18^{Ab}	76.39^{ab}	77.82^{a}	1.961	0.046
48 h	74.41^{ABb}	76.11^{ab}	77.07^{a}	1.886	0.044
96 h	73.56^{Bb}	75.49^{ab}	76.31^{a}	1.693	0.021
SEM	1.779	2.012	1.964	-	-
P-value	0.048	0.088	0.142	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

4.1.4 Meat color

Table 4-4 Effects of maternal VD_3 status on color of *longissimus* dorsi muscle in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
Lightness (L^*)					
24 h	59.82^{ABa}	57.02^{ABb}	56.59^{b}	1.393	0.035
48 h	61.08^{Aa}	58.96^{Ab}	56.83^{c}	1.931	0.003
96 h	58.80^{Ba}	56.14^{Bb}	56.01^{b}	1.883	0.021
SEM	2.032	2.261	3.412	-	-
P-value	0.046	0.039	0.225	-	-
Redness (a^*)					

Continuation of Table 4-4

Item	Groups			SEM	*P*-value
	LD	ND	HD		
24 h	7.08^{Ab}	8.24^{Aa}	8.31^{a}		
48 h	6.86^{ABb}	7.42^{ABa}	7.89^{a}	0.529	0.031
96 h	6.57^{Bb}	7.21^{Ba}	7.56^{a}	0.471	0.022
SEM	0.313	0.521	0.954	-	-
P-value	0.049	0.042	0.084	-	-
Yellowness (b^{*})					
24 h	8.35^{Ba}	7.54^{Bb}	7.28^{b}	0.484	0.011
48 h	8.87^{ABa}	7.89^{ABb}	7.37^{b}	0.583	0.035
96 h	9.29^{Aa}	8.56^{Ab}	7.81^{c}	0.618	0.003
SEM	0.554	0.602	0.658	-	-
P-value	0.047	0.033	0.096	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

Color of *longissimus dorsi* muscle in offspring pigs at different postmortem times was shown in Table 4-4. Offspring pigs from ND and HD groups had lower L* and b* values, and higher a* values compared with those from LD group at 24, 48 and 96 h postmortem storage, respectively ($P<0.05$). Meanwhile, L* values in offspring pigs from LD and ND groups were higher at 48 h than those at 96 h postmortem storage, and b* values were lower at 24 h than those at 96 h postmortem storage ($P<0.05$).

No differences in L* and b* values were observed in offspring pigs from HD group among all postmortem storage times ($P>0.05$). In addition, a* values in offspring pig from LD and ND group at 24 h were higher than those at 96 h postmortem storage, while no differences in a* values of offspring pigs from HD group were observed among all postmortem storage times ($P>0.05$).

4.1.5 LF-NMR

Distribution of the LF-NMR T_2 relaxation times of *longissimus dorsi* muscle in offspring pigs during postmortem storage was shown in Table 4-5 and Figure 4-1. No differences in T_{2a} relaxation times of offspring pigs from LD, ND and HD groups were observed at whole postmortem storage times (P>0.05). T_{21} relaxation times in offspring pigs from HD group were lower than those from LD group during postmortem storage times (P<0.05). At the same experimental group, no differences in T_{2a} and T_{21} relaxation times were found among all postmortem storage times (P>0.05). In addition, T_{22} relaxation times in offspring pigs from LD group were higher than those from ND and HD groups at whole postmortem storage times (P<0.05), and all groups had higher T_{22} relaxation times at 96 h compared with those at 24 and 48 h postmortem storage (P<0.05).

Table 4-5 Distribution of the LF-NMR T_2 relaxation times of *longissimus dorsi* muscle in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
T_{2a}, ms					
24 h	0.050^{a}	0.052^{a}	0.054^{a}	0.001	0.189
48 h	0.054^{a}	0.052^{a}	0.052^{a}	0.002	0.221
96 h	0.054^{a}	0.052^{a}	0.052^{a}	0.001	0.192
SEM	0.001	0.001	0.001	-	-
P-value	0.225	0.199	0.236	-	-
T_{21}, ms					
24 h	15.59^{a}	14.88^{ab}	14.18^{b}	0.631	0.047
48 h	14.98^{a}	14.32^{ab}	14.18^{b}	0.539	0.044
96 h	15.49^{a}	15.24^{ab}	14.88^{b}	0.524	0.043
SEM	1.244	1.162	1.385	-	-
P-value	0.053	0.074	0.099	-	-
T_{22}, ms					

Continuation of Table 4-5

Item	Groups			SEM	*P*-value
	LD	ND	HD		
24 h	254.08^{Ba}	220.98^{Bb}	220.98^{Bb}	3.616	0.022
48 h	233.27^{Ba}	200.92^{Bb}	200.92^{Bb}	2.948	0.027
96 h	307.37^{Aa}	285.50^{Ab}	242.55^{Ac}	5.592	0.003
SEM	8.965	9.887	10.02	-	-
P-value	0.022	0.033	0.048	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$). T_{2a}, binding water relaxation. T_{21}, immobile water relaxation time. T_{22}, free water relaxation time.

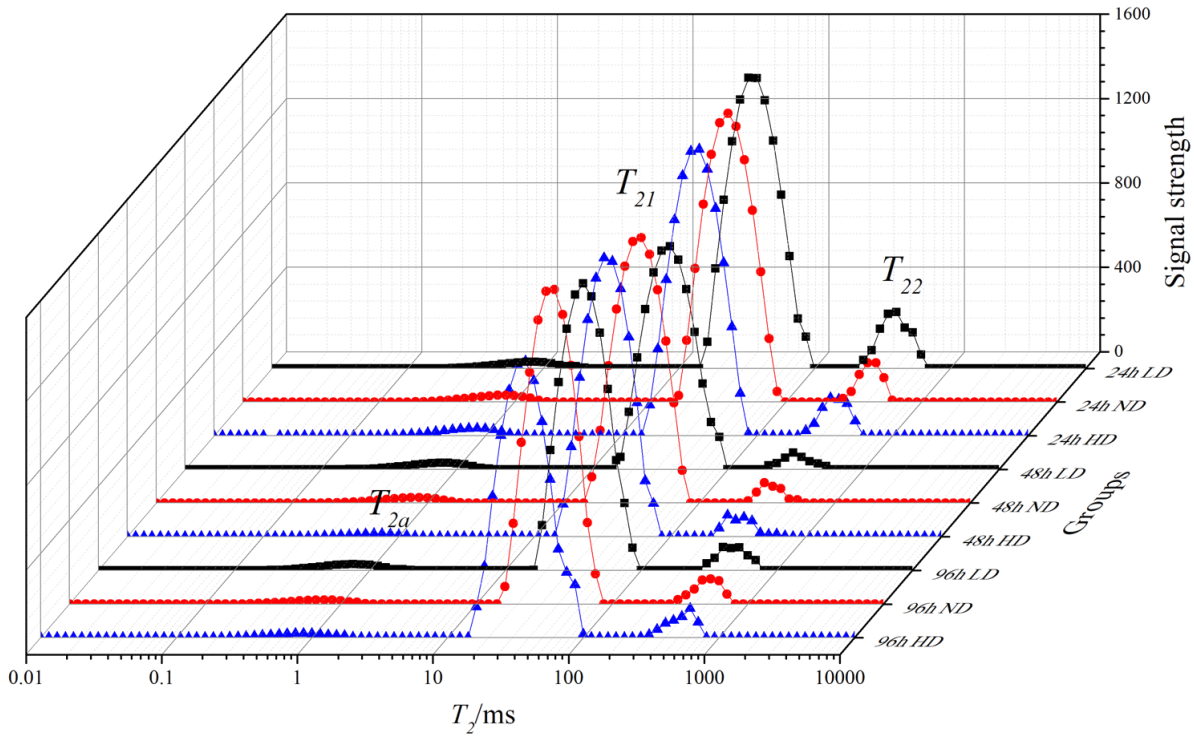


Figure 4-1 Three-dimensional T_2 relaxation time plot of *longissimus dorsi* muscle in offspring pigs from LD, ND and HD groups during postmortem storage

4.1.6 Discussion

Our present research found that there were interactions between maternal VD_3 supplementation and postmortem storage on pH, shear force, WHC, meat color and distribution of the LF-NMR T_2 relaxation times of *longissimus dorsi* muscle in offspring pigs for the first time. These results indicated that maternal VD_3 status and postmortem storage affected meat quality attributes in offspring pigs by regulating meat quality index.

Previous study found that the decrease of pH value is due to the accumulation of lactic acid produced by anaerobic glycolysis [199]. In this study, pH values of LD and ND group were significantly decreased with postmortem storage time, while no differences in pH values of HD group were obtained. These results suggested that maternal VD_3 supplementation could maintain the relative stability of pH value of *longissimus dorsi* muscle in offspring pigs at 24, 48 and 96 h postmortem storage. Our findings indicated that similar metabolic changes occurred in *longissimus dorsi* muscle of offspring pigs from HD group during postmortem storage times.

In the present study, shear force of offspring pigs from HD group was lower than that from ND and LD groups, which indicated that maternal VD_3 status influenced shear force of *longissimus dorsi* muscle in offspring pigs. Our previous studies have observed that maternal VD_3 supplementation increased IMF accumulation of *longissimus dorsi* muscle in offspring piglets via regulating adipogenic genes expression [21, 22]. IMF in skeletal muscle is positively correlated with meat tenderness [185]. Therefore, maternal VD_3 supplementation decreased shear force of *longissimus dorsi* muscle in offspring pigs may be due to the increasing IMF content. Previous study also found that VD_3 increased tenderness of *longissimus dorsi* muscle in beef through activation of m-calpain [203]. Whereas, its mechanism needs to be still identified in further studies. Meanwhile, shear force of values in HD group greatly decreased after 96 h postmortem, while no significant differences in shear force of LD and ND groups were found during postmortem storage. These results indicated that maternal VD_3 supplementation could rapidly decline shear force in offspring pigs, and improved pork palatability during postmortem storage. Similar result was reported by previous

researcher [210], who observed that the shear force values of chicken breast muscle greatly decreased after 5 d postmortem. In addition, the chilling process also influenced tenderness of pork muscle *longissimus* dorsi [217]. Whereas, other study noted that shear force of chicken breast muscles was significantly increased during storage [210]. Inconsistent research results in shear force might be due to different species, postmortem storage time, experimental conditions and storage temperature.

WHC affects economic and sensory properties of meat, which is very important in the meat industry [197]. Previous studies showed that the WHC is very important for reducing exudates, and improving palatability, juiciness, tenderness, and meat quality attributes [198, 247]. Meanwhile, the juiciness of meat is correlated to pH value and IMF content of pork. pH decline affects WHC, moisture, tenders and color of meat [248]. In this study, HD group had higher pH value and WHC compared with LD group during postmortem storage times. These results suggested that pH value were negatively correlated with exudates, and positively correlated with WHC of meat [198]. In addition, HD group had no differences in pH values and WHC of *longissimus dorsi* muscle at 24, 48, and 96 h postmortem storage, which indicated that maternal VD_3 supplementation could inhibited the decline of WHC, and improved juiciness and eating meat quality attributes of *longissimus dorsi* muscles in offspring pigs during postmortem storage. Postmortem storage decreased WHC of *longissimus dorsi* muscle in offspring pigs from LD group. These results indicated that maternal VD_3 deficiency could significantly decreased juiciness of meat and meat quality attributes in offspring pigs during postmortem storage times. Similar results found that WHC values decreased during storage time and increased drip loss in packaged meat, which decreased meat quality [249].

Meat color is usually used for assessing freshness and meat quality attributes [189], which is the direct factor that determine consumers' purchase [188]. In this study, offspring pigs from HD group had lower L*, b* values, and higher a* value of *longissimus dorsi* muscle compared with those from LD group during postmortem storage times, which improved red color and decreased lightness and yellowness of meat. In addition, the *longissimus dorsi* muscle of offspring pigs in HD group tended

to be darker appearance than those in LD and ND groups during postmortem storage. These results suggested that maternal VD_3 supplementation could improve meat quality attributes of offspring pigs. Previous study found that the dark appearance of meat color may be caused by metmyoglobin reduction [250]. Other study noted that inactivation enzyme systems are considered to contribute to the dark appearance of meat color [251]. Certainly, the mechanism underlying still needs to be proved by further investigation. Meanwhile, L* and a* values of *longissimus dorsi* muscle in offspring pigs from LD and ND groups were decreased significantly with postmortem storage time, while b* values increased with postmortem storage time, which suggested that maternal VD_3 supplementation could inhibit the decline of lightness and redness, and the rise of yellowness in *longissimus dorsi* muscle of offspring pigs during postmortem storage. These results indicated that maternal VD_3 supplementation could prolong the postmortem storage time and retail display of *longissimus dorsi* muscle in offspring pigs.

The moisture content (or WHC) is directly related to the color, tenderness, juiciness, and flavor of meat, which affects the preservation and processing characteristics of meat. In addition, the moisture content and distribution in meat showed a dynamic change process during storage and affected the meat quality [252]. LF-NMR relaxation time can be used to characterized water distribution and mobility in meat, and it is helpful to understand the influence of postmortem process, cooling and other factors on WHC of meat [218]. Several studies have reported that the LF-NMR relaxation times reflects the degree of tightness between water and substrate, which could distinguish water interacting with macromolecules, water in myofibrils and reticular tissue, as well as extracellular water in meat [195]. Meanwhile, T_{2a} (0-10 ms) represents the binding water combined with macromolecules substances such as protein, T_{21} (10-100 ms) represents water that is existed in the myofibrillar network of muscle, and T_{22} (100-400 ms) represents free water between fiber bundles of muscle [253, 254]. In this present study, at the same experimental group no differences in T_{2a} of *longissimus dorsi* muscle in offspring pigs were observed during postmortem storage times. These results suggested that maternal VD_3 status had no impact on binding water

of muscle in offspring pigs during postmortem storage. Meanwhile, T_{21} and T_{22} of *longissimus dorsi* muscle of offspring pigs from HD group were lower than those from LD group during whole postmortem storage, which suggested that maternal VD_3 supplementation decreased free water content and water mobility in *longissimus dorsi* muscle of offspring pigs during postmortem storage. These results indicated that offspring pigs from HD group had higher WHC and juiciness in *longissimus dorsi* muscle, and better meat quality attributes compared with LD group. Our study also found that T_{22} of *longissimus dorsi* muscle in offspring pigs form all groups increased with postmortem storage time. Whereas, at the same experimental group no differences in T_{2a} and T_{21} of *longissimus dorsi* muscle of offspring pigs were observed during postmortem storage times. These results revealed that water mobility was affected, while bounded water wasn't influenced by postmortem storage times.

Taken together, our findings indicated that meat quality attributes, water state and distribution in offspring pigs were affected by maternal VD_3 status during pregnancy and postmortem storage. Meanwhile, HD group had better meat quality attributes (lower shear force, L*, b* values, T_{21}, T_{22}, and higher pH, WHC, a* value) compared with LD and ND groups during postmortem storage times.

4.2 Effects of maternal vitamin D_3 on meat quality technological properties in offspring pigs during frozen storage

4.2.1 Meat color

Table 4-6 Changes in color of *longissimus dorsi* muscle in offspring pigs during frozen storage

Item	Frozen storage time (h)					S.E.M	*P*-value
	0	24	48	72	96		
*L**							
HD	54.14 ^{bC}	56.75 ^{aC}	56.98 ^{aC}	57.22 ^{aC}	57.22 ^{aC}	1.18	0.031
ND	56.97 ^{bB}	57.18 ^{abB}	59.06 ^{aB}	60.14 ^{aB}	60.64 ^{aB}	0.76	0.022
LD	59.65 ^{bA}	59.94 ^{abA}	61.22 ^{aA}	61.55 ^{aA}	61.63 ^{aA}	0.41	0.016
S.E.M	0.87	1.07	0.79	0.61	0.77	-	-

Continuation of Table 4-6

Item	Frozen storage time (h)					S.E.M	*P*-value
	0	24	48	72	96		
P-value	0.015	0.027	0.011	0.009	0.012	-	-
*a**							
HD	8.23 ^{aA}	8.28 ^{aA}	7.66 ^{abA}	7.48 ^{abA}	7.35^{bA}	0.13	0.002
ND	8.11 ^{aA}	8.16 ^{aA}	7.31 ^{bA}	7.21 ^{bA}	7.08^{bA}	0.14	0.004
LD	7.26 ^{aB}	7.04 ^{aB}	6.65 ^{abB}	6.52 ^{abB}	6.34^{bB}	0.15	0.007
S.E.M	0.14	0.10	0.13	0.14	0.12	-	-
P-value	0.003	0.002	0.004	0.005	0.002	-	-
*b**							
HD	5.85 ^{cC}	7.32 ^{bC}	7.49 ^{bC}	7.74 ^{abC}	7.95 ^{aC}	0.12	0.002
ND	6.23 ^{cB}	7.66 ^{bB}	7.95 ^{abB}	8.35 ^{aB}	8.67 ^{aB}	0.14	0.006
LD	7.95 ^{cA}	8.45 ^{bA}	8.98 ^{abA}	9.22 ^{aA}	9.40 ^{aA}	0.16	0.001
S.E.M	0.14	0.15	0.13	0.13	0.14	-	-
P-value	0.009	0.003	0.008	0.009	0.004	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

As shown in Table 4-6, L* and b* value of *longissimus dorsi* muscle in offspring pigs born to all groups increased with frozen storage time. At 0 h, L* and b* value of HD, ND and LD groups were 51.14, 56.97, 59.65 and 5.85, 6.23, 7.95, and at 96 h increased to 57.58, 60.64, 61.63 and 7.95, 8.67, 9.40 respectively ($P<0.05$). L* and b* value of ND group was lower than that of LD group, and was higher that of HD group during frozen storage. a* value of all groups decreased with frozen storage time, a* value of HD, ND and LD groups were 8.23, 8.11, 7.26 at 0 h, and decreased to 7.35, 7.08, 6.34 respectively ($P<0.05$). The a* values of LD group were lower than that of ND and HD groups during frozen storage ($P<0.05$).

4.2.2 Thawing loss, cooking loss and shear force

Table 4-7 Changes in thawing loss, cooking loss and shear force of *longissimus dorsi* muscle in offspring pigs during frozen storage

Item	Frozen storage time (h)					S.E.M	*P*-value
	0	24	48	72	96		
Thawing loss, %							
HD	1.64 ^{eC}	1.78 ^{dC}	1.93 ^{cC}	2.13 ^{bC}	2.281^{aC}	0.17	0.009
ND	2.11 ^{eB}	2.31 ^{dB}	2.44 ^{cB}	2.65 ^{bB}	2.94 ^{aB}	0.18	0.011
LD	2.35 ^{eA}	2.57 ^{dA}	2.81 ^{cA}	3.12 ^{bA}	3.38 ^{aA}	0.21	0.014
S.E.M	0.13	0.16	0.14	0.21	0.23	-	-
P-value	0.012	0.015	0.021	0.023	0.019	-	-
Cooking loss, %							
HD	28.84 ^{bB}	29.29 ^{abB}	30.56 ^{aB}	31.22 ^{aB}	32.04 ^{aB}	1.29	0.006
ND	31.88 ^{bA}	32.01 ^{abA}	32.88 ^{abA}	33.48 ^{aA}	34.18 ^{aA}	0.85	0.008
LD	32.66 ^{bA}	32.94 ^{abA}	33.24 ^{abA}	33.89 ^{aA}	34.76 ^{aA}	0.95	0.004
S.E.M	0.65	1.03	1.06	1.10	0.98	-	-
P-value	0.024	0.031	0.028	0.016	0.011	-	-
Shear force, kg							
HD	2.86 ^{aC}	2.77 ^{aC}	2.53 ^{abC}	2.46 ^{bC}	2.42 ^{bC}	0.11	0.003
ND	3.31 ^{aB}	3.14 ^{aB}	3.02 ^{abB}	2.99 ^{bB}	2.98 ^{bB}	0.22	0.004
LD	3.58 ^{aA}	3.46 ^{aA}	3.38 ^{abA}	3.42 ^{aA}	3.26 ^{bA}	0.16	0.006
S.E.M	0.21	0.17	0.15	0.14	0.14	-	-
P-value	0.008	0.006	0.007	0.005	0.003	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$).
In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

As shown in Table 4-7, thawing loss and cooking loss of *longissimus dorsi* muscle in offspring pigs born to all groups increased with frozen storage time ($P<0.05$). Whereas, shear force of all groups decreased with frozen storage time ($P<0.05$).

Thawing loss and shear force of ND group was lower than that of LD group, while was higher than that of HD group during frozen time, respectively (P<0.05). Meanwhile, cooking loss of HD group was lower than that of ND and LD groups during frozen storage (P<0.05).

4.2.3 LF-NMR relaxation time

Table 4-8 Changes in LF-NMR relaxation times of *longissimus dorsi* muscle in offspring pigs during frozen storage

Item	Frozen storage time (h)					S.E.M	*P*-value
	0	24	48	72	96		
T_{2a}, ms							
HD	0.50^{aA}	0.55^{aA}	0.53^{aA}	0.54^{aA}	0.54^{aA}	0.003	0.318
ND	0.50^{aA}	0.53^{aA}	0.54^{aA}	0.55^{aA}	0.55^{aA}	0.004	0.254
LD	0.50^{aA}	0.512^{aA}	0.56^{aA}	0.56^{aA}	0.56^{aA}	0.002	0.281
S.E.M	0.003	0.002	0.002	0.003	0.003	-	-
P-value	0.225	0.231	0.188	0.197	0.332	-	-
T_{21}, ms							
HD	13.56^{bB}	15.22^{abB}	15.58^{abB}	15.96^{aB}	16.14^{aB}	1.02	0.041
ND	14.17^{bA}	15.99^{abA}	16.42^{abA}	16.81^{aA}	16.94^{aA}	0.87	0.035
LD	14.18^{bA}	16.14^{abA}	16.38^{abA}	16.89^{aA}	17.21^{aA}	0.73	0.046
S.E.M	1.05	0.88	0.81	0.74	0.84	-	-
P-value	0.047	0.039	0.028	0.033	0.024	-	-
T_{22}, ms							
HD	167.17^{bC}	230.32^{abB}	234.45^{abB}	241.18^{aC}	248.47^{aC}	7.19	0.048
ND	183.48^{cB}	231.16^{bB}	235.09^{bB}	269.95^{abB}	291.18^{aB}	9.89	0.044
LD	192.20^{cA}	262.62^{bA}	272.23^{bA}	301.35^{abA}	324.42^{aA}	9.78	0.038
S.E.M	5.78	9.34	10.30	10.71	8.64	-	-
P-value	0.026	0.041	0.046	0.029	0.031	-	-

In the same column, values with different capital superscripts mean significant difference (P<0.05). In the same line, values with different small letter superscripts mean significant difference (P<0.05).

As shown in Table 4-8 and figure 4-2, T_{21} and T_{22} relaxation times of *longissimus dorsi* muscle in offspring pigs born to all groups increased with frozen storage, whereas, no differences in T_{2a} relaxation times of all groups were observed with frozen storage (P>0.05). T_{21} relaxation times of HD group was lower than that of ND and LD groups (P<0.05). Meanwhile, T_{22} relaxation times of ND group was lower than that of LD group, and was higher than that of HD group during frozen storage, respectively (P<0.05). There was no difference in T_{2a} relaxation times among HD, ND and LD groups during frozen storage (P>0.05).

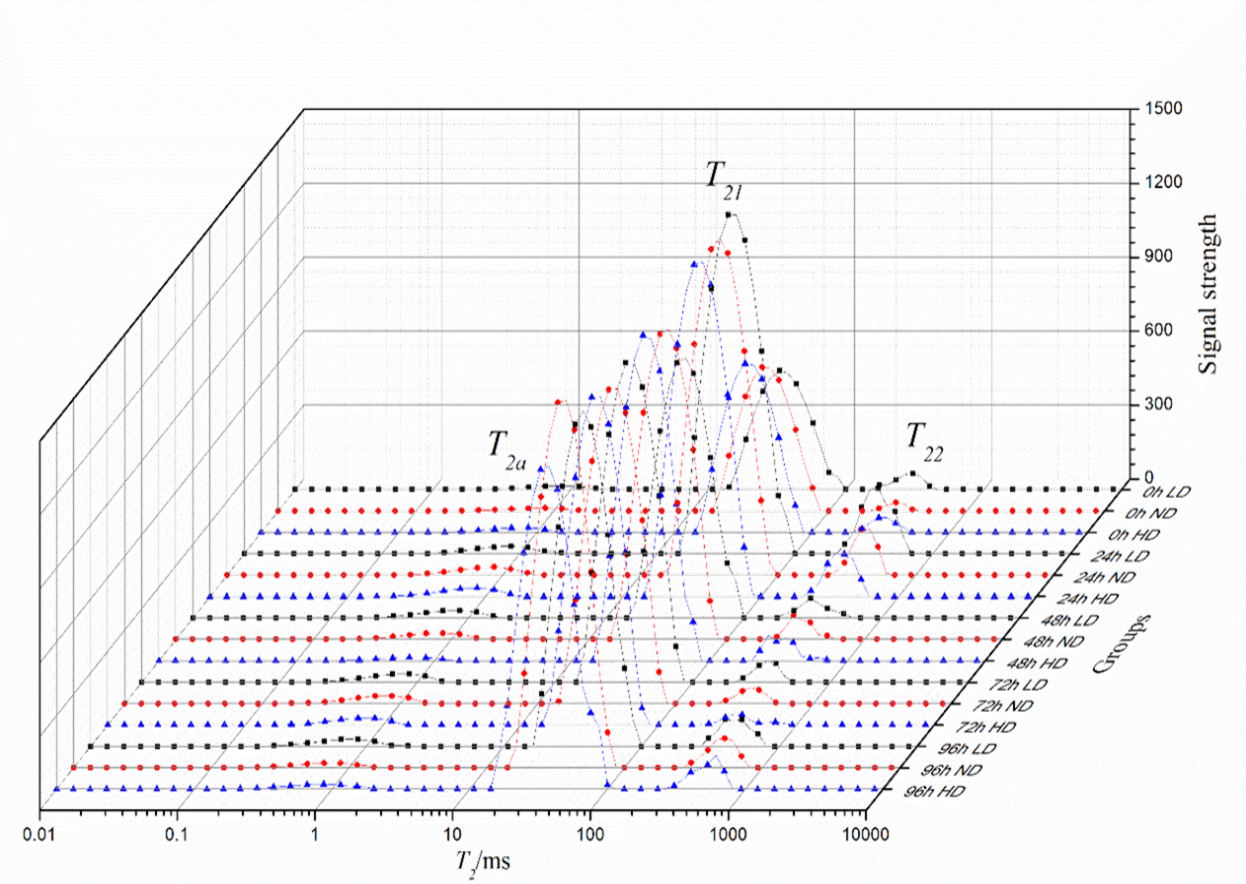


Figure 4-2. Three-dimensional T_2 relaxation time plot of *longissimus dorsi* muscle in offspring pigs from LD, ND and HD groups during frozen storage

4.2.4. Discussion

Meat color usually affects consumer's purchasing decisions, and is used for assessing freshness and attributes of meat quality [189]. In this study, L* and b* values of

longissimus dorsi muscle in offspring pigs born to all groups were increased with the prolongation of frozen storage time. Whereas, a* value decreased with extended frozen storage time. These results suggested that meat color was affected by frozen storage time. Similar results were observed by previous researcher [219], who found that meat color of porcine *longissimus dorsi* muscle was influenced by frozen storage time, and a* value decreased with increasing frozen storage time, whereas, the change trend of L* and b* values were opposite to that of a* value. Other researcher also found that there is a negative correlation between the a* value of frozen muscle and frozen storage time. These results may be due to the decrease metmyoglobin-reducing enzymes activity with prolonged frozen storage [255]. Meanwhile, L* value increased with frozen storage time. These results may be due to the increase of muscle fiber contraction during freezing, which reduced the light scattering on the meat surface [256]. In this study, b* value also increased with frozen storage time. Similar results were reported by previous researcher [219], which may be related to increase in protein oxidation and thiobarbituric acid-reactive substance during frozen storage.

In this study we found that compared with HD group, LD group had higher L^* and b^* values, while lower a^* values of *longissimus dorsi* muscle at whole frozen storage periods. These results indicated that maternal VD_3 status may influenced meat color of frozen muscle in offspring, and maternal VD_3 deficiency decreased sensory quality of meat. Previous study found that pork color discoloration is associated with pigment and lipid oxidation [191]. Our results may be due to maternal VD_3 status changed the activity of the oxidative processes and pigment oxidation in *longissimus dorsi* muscle, which influenced a^*, L^* and b^* values of pork of offspring pigs. Similar results were observed by previous researcher, who found that dietary VD_2 decreased lipid peroxidation of *longissimus* thoracis steak of pigs, improved redness value, and color stability [201]. Whereas, other study found that supplementation of VD_3 did not influence a^* and b^* value, while decreased L^* value of pork in finishing pigs [200]. Inconsistent research results in meat color might be due to species, ages, dosage, and duration of feeding VD_3.

Freezing and thawing usually influenced the amount of thawing loss and drip loss, and when the freezing time was more than 19.5 min, the amount of thawing loss and drip loss was significantly higher than that before freezing [193]. Previous study demonstrated that thawing loss usually affects the color and sensory quality of meat [194], and is associated with the destruction of muscle fiber structure and the denaturation of protein [193]. In this present study, the thawing loss of all groups increased with frozen storage, which may be due to the fracture of muscle fibers was caused by the formation of ice crystals at frozen storage [257]. These results suggested that thawing loss of *longissimus dorsi* muscle in offspring pigs were affected by frozen storage time. Similar results were observed by previous report [219], who found that thawing loss of porcine *longissimus dorsi* muscle (air freezing, immersion freezing and ultrasound-assisted immersion freezing) increased with frozen storage time. Meanwhile, our results found that thawing loss in offspring pigs born to LD group were significantly higher than that born to HD group for the first time, which indicated that maternal VD_3 supplementation may reduce the formation of ice crystals, and decrease damage of the muscle structure during frozen storage, and improve WHC and meat quality attributes in offspring pigs. In addition, maternal VD_3 deficiency increased destruction of muscle structure and decreased the water binding capacity of muscle in offspring pigs. These results indicated that after thawing, compared with LD group, the muscle samples of HD group had stronger ability to reabsorb melted water back into the cells. Whereas, its regulatory mechanism still needs to be further studied.

Cooking loss is generally considered to be the release of chemically bound water due to fat melting and protein denaturation during cooking [192]. There is a negative correlation between cooking loss and eating quality of meat [182]. In this study, cooking loss of *longissimus dorsi* muscle of all groups increased with frozen storage time. These results suggested that the quality of meat still be lost during the process of frozen storage. Similar results were observed by previous researcher [219], who found that cooking loss of porcine *longissimus dorsi* muscle significantly increased with increase in frozen storage time. Meanwhile, HD group had the lowest cooking loss during frozen storage, which indicated that maternal VD_3 supplementation could prolong and

protect the porcine meat quality through inhibiting the decrease in cooking loss of *longissimus dorsi* muscle in offspring pigs. The reason may be supposed that maternal VD_3 supplementation maintained the integrity of muscle tissue and decreased cooking loss in offspring pigs. However, the mechanism underlying still needs to be investigated by further.

Shear force usually reflects the tenderness of meat, and the increase of tenderness is associated to the length of frozen storage [193]. In the present study, shear force of *longissimus dorsi* muscle of all groups decreased with frozen storage time. Similar results were observed by previous researcher [198], who found that shear force of freeze-thawed pork decreased with storage time. Meanwhile, some researchers also observed that shear force of beef decreased with frozen storage [258, 259]. Whereas, other researcher found that freezing did not influenced shear force of beef [192]. Inconsistent research results in shear force value might be due to species, frozen storage time and temperature. Previous results demonstrated that shear force is negatively correlated to IMF content of muscle [185]. In this study, shear force in offspring pigs born to LD group were significantly higher than that born to HD group, which suggested that maternal VD_3 deficiency maybe decreased the tenderness of *longissimus dorsi* muscle in offspring pigs during frozen storage through inhibiting the formation of IMF [21]. These results indicated that maternal VD_3 status could affect the meat quality attributes in offspring pigs during frozen storage.

LF-NMR can reflect the distribution and migration of water in meat products, and T_{2a} represents the bound water, T_{21} corresponds to the immobilized water, as well as T_{22} represents the free water [260]. In this study, no differences in T_{2a} relaxation time of all groups were observed with frozen storage time. Whereas, T_{21} and T_{22} relaxation times of all groups increased with frozen storage. These results suggested that the migration and distribution of water was affected by frozen storage, and frozen storage could lead to a certain level of the immobile water shifting to free water. Similar results were reported by previous researcher, who observed that T_{21} relaxation times of porcine *longissimus* muscle increased with increased freeze-thaw cycles [196], which indicated that frozen storage could reduce the abundance of water in *longissimus dorsi* muscle.

Other study also reported that T_{21} relaxation times of hake muscle increased with frozen storage [261]. These results may be due to the formation of ice crystals during frozen storage, which destroy the physical structure of muscle tissue, and resulting in the conversion of partially immobilized water into free water [193]. Previous results found that relaxation time is correlated to meat quality attributes, and higher T_2 relaxation time usually reflects higher thawing loss [219, 262]. Our results also found that the change in T_2 relaxation times is similar to that of the thawing loss during frozen storage. In addition, T_{21} and T_{22} relaxation times in offspring pigs born to HD group were significantly lower than that born to LD group, which indicated that maternal VD_3 supplementation could increase WHC and meat quality in offspring pigs. Whereas, maternal VD_3 deficiency increased thawing loss and decreased WHC of offspring pigs during frozen storage.

4.3 Effects of maternal vitamin D_3 status on quality and technological properties of pork batters in offspring pigs during cold storage

4.3.1 Cooking loss

Table 4-9 Effects of maternal VD_3 status on cooking loss of pork batters in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
0 h	13.38^{Ba}	12.61^{Ba}	10.29^{b}	0.693	0.043
24 h	14.66^{ABa}	13.84^{ABa}	11.37^{b}	0.838	0.031
48 h	15.76^{Aa}	14.96^{Aa}	11.63^{b}	0.561	0.026
SEM	0.876	0.932	0.541	-	-
P-value	0.034	0.047	0.066	-	-

In the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

As shown in Table 4-9, cooking loss of pork batters in offspring pigs born to HD group was lower than those born to ND and LD groups at 0, 24 and 48 h postmortem storage ($P<0.05$), while there were no differences in cooking loss between LD and ND

groups (P>0.05). Meanwhile, offspring pigs born to LD and ND groups had lower cooking loss of pork batters at 0 h compared with that at 48 h postmortem storage (P<0.05). No significant differences in cooking loss of pork batters of offspring pigs born to HD group were found among all postmortem storage times (P>0.05).

4.3.2 Color

Table 4-10 Effects of maternal VD_3 status on color of pork batters in offspring pigs during postmortem storage

Item	Groups			SEM	P-value
	LD	ND	HD		
Lightness (L^*)					
0 h	79.44^{Aa}	78.72^{Aab}	75.14^{b}	1.811	0.045
24 h	78.34^{ABa}	77.63^{ABab}	75.60^{b}	2.193	0.039
48 h	77.06^{Ba}	75.54^{Bab}	74.83^{b}	1.547	0.041
SEM	1.633	1.766	2.441	-	-
P-value	0.027	0.036	0.077	-	-
Redness (a^*)					
0 h	4.96^{Ab}	5.08^{Aab}	5.37^{a}	0.152	0.013
24 h	3.61^{ABb}	3.96^{ABab}	4.66^{a}	0.339	0.048
48 h	3.42^{Bb}	3.67^{Bab}	4.32^{a}	0.207	0.024
SEM	0.293	0.344	0.413	-	-
P-value	0.022	0.018	0.053	-	-
Yellowness (b^*)					
0 h	9.51^{Ba}	8.33^{Bb}	8.18^{b}	0.352	0.043
24 h	9.98^{ABa}	8.91^{ABb}	8.63^{b}	0.579	0.032
48 h	10.87^{Aa}	9.73^{Ab}	8.84^{b}	0.424	0.015
SEM	0.873	0.698	0.732	-	-
P-value	0.036	0.013	0.061	-	-

In the same column, values with different capital superscripts mean significant difference (P<0.05).
In the same line, values with different small letter superscripts mean significant difference (P<0.05).

4.3.3 TPA

Table 4-11 Effects of maternal VD_3 status on TPA of pork batters in offspring pigs during postmortem storage

Item	Groups			SEM	*P*-value
	LD	ND	HD		
Hardness, N					
0 h	38.19^{Ac}	43.39^{Ab}	50.15^{Aa}	1.873	0.041
24 h	33.20^{Bc}	37.33^{Bb}	42.74^{Ba}	1.795	0.045
48 h	33.14^{Bc}	35.84^{Bb}	37.03^{Ca}	1.532	0.038
SEM	1.006	1.704	1.014	-	-
P-value	0.025	0.029	0.012	-	-
Springiness					
0h	0.941^{Ab}	1.189^{Aab}	1.360^{Aa}	0.004	0.003
24h	0.866^{Bb}	0.930^{Bab}	1.051^{Ba}	0.006	0.018
48 h	0.813^{Bb}	0.828^{Bab}	0.841^{Ca}	0.012	0.027
SEM	0.011	0.063	0.019	-	-
P-value	0.023	0.034	0.012	-	-
Cohesiveness					
0 h	0.491^{Ab}	0.539^{Aab}	0.599^{Aa}	0.009	0.004
24 h	0.416^{Bb}	0.436^{Bab}	0.480^{Ba}	0.004	0.003
48 h	0.414^{Bb}	0.425^{Bab}	0.449^{Ca}	0.006	0.013
SEM	0.034	0.029	0.014	-	-
P-value	0.031	0.022	0.004	-	-
Chewiness, N·mm					
0 h	15.45^{Ac}	19.63^{Ab}	23.68^{Aa}	0.554	0.022
24 h	10.15^{Bc}	13.57^{Bb}	18.58^{Ba}	0.538	0.043
48 h	9.75^{Bc}	12.09^{Bb}	14.53^{Ca}	0.117	0.048

Continuation of Table 4-11

Item	Groups			SEM	*P*-value
	LD	ND	HD		
SEM	0.884	0.691	1.135	-	-
P-value	0.033	0.025	0.011	-	-

erwIn the same column, values with different capital superscripts mean significant difference ($P<0.05$). In the same line, values with different small letter superscripts mean significant difference ($P<0.05$).

As shown in Table 4-10, pork batters of offspring pigs born to HD group had higher a^* values, and lower L^* values compared with those born to LD group at 0, 24 and 48 h postmortem storage ($P<0.05$). In addition, L^* and a^* values of pork batters in offspring pigs from LD and ND groups were higher at 0 h than those at 48 h postmortem storage ($P<0.05$).

However, no significant differences in L^* and a^* values of pork batters were determined in offspring pigs born to HD group among 0, 24 and 48 h postmortem storage ($P>0.05$). b^* value of pork batters in offspring from ND and HD groups were lower than those from LD group at 0, 24 and 48 h postmortem storage ($P<0.05$). Meanwhile, b^* values of pork batters in offspring pigs born to LD and ND groups at 48 h were higher than those at 0 h postmortem storage ($P<0.05$), while no significant differences in b^* values of pork batters of offspring pigs born to HD group were measured among all postmortem storage times ($P>0.05$).

As shown in Table 4-11, hardness, and chewiness of pork batters in offspring pigs from ND group were lower than those from HD group, while higher than those from LD group at 0, 24 and 48 h postmortem cold storage ($P<0.05$). Meanwhile, springiness and cohesiveness of pork batters in offspring pigs from HD group were higher than those from LD group during all postmortem cold storage times ($P<0.05$). In addition, offspring pigs from all groups had higher hardness, springiness, cohesiveness, and chewiness of pork batters at 0 h compared with those at 24 and 48 h postmortem cold storage ($P<0.05$).

4.3.4 LF-NMR

As shown in Table 4-12 and Figure 4-3, there were three characteristic peaks in the T_2 relaxation time map of pork batters in offspring pigs. There were no significant differences in T_{2a} relaxation time of pork batters among LD, ND and HD groups during the whole postmortem storage time (P>0.05).

Table 4-12 Effects of maternal VD_3 status on LF-NMR of pork batters in offspring pigs during postmortem storage

Item	Groups			SEM	P-value
	LD	ND	HD		
T_{2a}, ms					
0 h	0.054^{a}	0.054^{a}	0.052^{a}	0.001	0.207
24 h	0.052^{a}	0.054^{a}	0.052^{a}	0.001	0.173
48 h	0.054^{a}	0.054^{a}	0.052^{a}	0.001	0.196
SEM	0.001	0.001	0.001	-	-
P-value	0.211	0.198	0.242	-	-
T_{21}, ms					
0h	15.19^{a}	14.88^{ab}	13.25^{b}	0.801	0.039
24h	15.47^{a}	14.88^{ab}	14.68^{b}	0.663	0.022
48 h	14.88^{a}	14.48^{ab}	13.68^{b}	0.512	0.047
SEM	0.713	0.601	0.858	-	-
P-value	0.061	0.274	0.127	-	-
T_{22}, ms					
0 h	231.01^{a}	191.95^{Bb}	191.46^{Bb}	5.416	0.025
24 h	232.52^{a}	193.51^{Bb}	192.20^{Bb}	3.426	0.031
48 h	233.76^{a}	205.95^{Ab}	200.92^{Ab}	6.191	0.029
SEM	9.853	6.627	5.032	-	-
P-value	0.092	0.046	0.041	-	-

In the same column, values with different capital superscripts mean significant difference (P<0.05).
In the same line, values with different small letter superscripts mean significant difference (P<0.05).

Whereas, T21 relaxation time of pork batters of offspring pigs in HD group at 0, 24 and 48 h postmortem were significantly lower than that in LD groups (P<0.05). In addition, T22 relaxation time of pork batters of offspring pigs in LD group were higher than that in ND and HD groups at 0, 24 and 48 h (P<0.05).

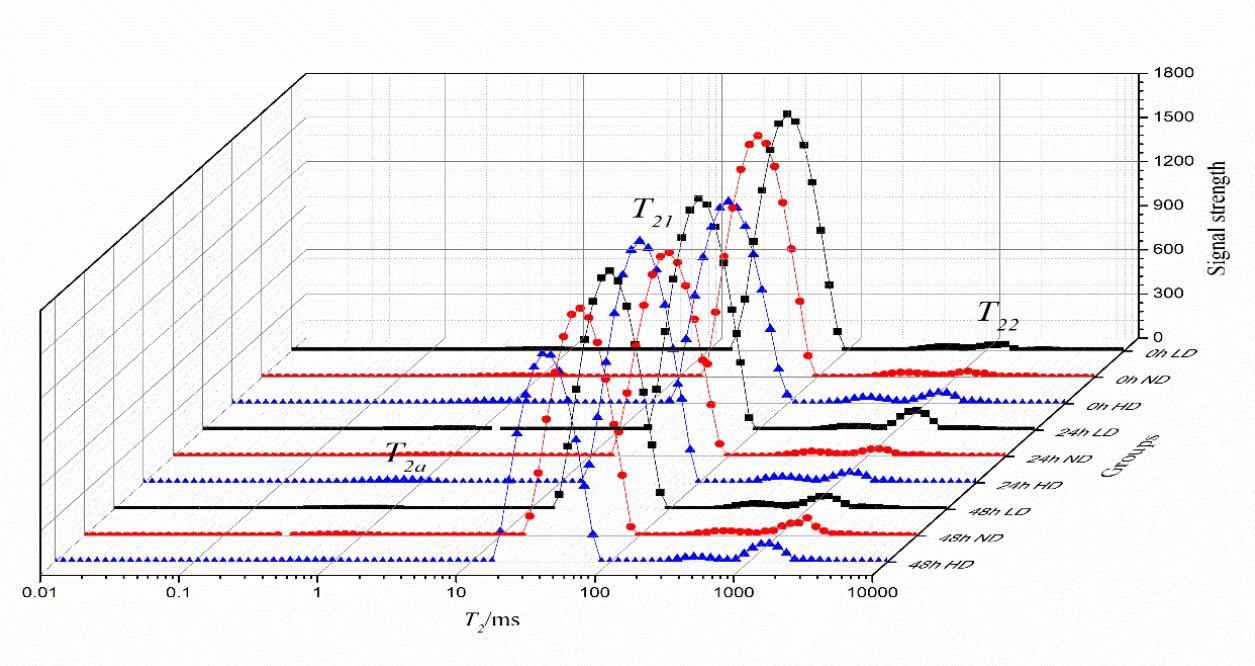


Figure 4-3. Three-dimensional T_2 relaxation time plot of pork batters in offspring pigs from LD, ND and HD groups during postmortem storage T_{2a}, binding water relaxation. T_{21}, immobile water relaxation time. T_{22}, free water relaxation time.

Meanwhile, The T_{22} relaxation time of pork batters of offspring pigs in all groups at 48 h was significantly higher than that at 0 and 24 h postmortem cold storage (P<0.05). No differences in T_{21} relaxation time of pork batters were also found at 0, 24 and 48 h postmortem storage in the same group (P>0.05).

4.3.5 Discussion

In the present study, it was found that there was an interaction between maternal VD_3 status and postmortem storage on cooking loss, meat color, texture properties, LF-NMR T_2 relaxation time and dynamic rheological properties of pork batters in offspring pigs. Results revealed that maternal VD_3 level and postmortem storage impacted the quality of pork batters in offspring pigs by adjusting meat quality indices.

Our research found that the cooking loss of LD and ND groups was lower at 0 h than at 48 h postmortem storage. These results suggested that WHC of pork batters was affected by postmortem cold storage. These results are in accordance with previous reports [263], in which it was found that freezing or frozen storage decrease the WHC of meat, and the cooking loss of meat was increased during cold storage [217, 264]. These results may be due to the destruction of muscle tissue structure during postmortem cold storage, resulting in the reduction of water protein interaction, which reduces the WHC of meat [265]. Previous study has demonstrated that the decrease in WHC of meat may be related to the decrease of pH value, the destruction of muscle fiber structure and the denaturation of proteins during cold storage [193]. Meanwhile, no differences in cooking loss of pork batters were observed in HD group during whole postmortem storage times, which suggested that maternal VD_3 addition in high dose decreased the cooking loss, and increased juiciness of pork batters in offspring pigs during postmortem storage times.

The reason may be that maternal VD_3 supplementation in high dose inhibited the decline of cooking loss by reducing the decline of pH value in meat samples, and improving the juiciness of pork batters in offspring pigs[266]. In addition, the cooking loss of HD group was lower than that of LD and ND groups at 0 h, 24 h and 48 h postmortem, which indicated that maternal VD_3 supplementation in high dose could improve the water retention of pork batters of offspring pigs. The reason may be that maternal VD_3 supplementation in high dose decrease cooking loss of offspring pigs by regulating pH values, muscle fiber structure and the denaturation of proteins during postmortem cold storage [193, 266].

Growing evidence have demonstrated that one of the most important sensory attributes of fresh pork is color, which affects consumers' purchase of red meat [265, 267]. In this study, offspring pigs from HD group had higher a^* values of pork batters than the offspring pigs from LD group at 0, 24 and 48 h postmortem cold storage. These results revealed that maternal VD_3 supplementation in high dose can maintained the stability of redness of *longissimus dorsi* muscle in offspring pigs.

These results are consistent with the color change trend of *longissimus dorsi* muscle in offspring pigs during postmortem storage times [266]. The reason may be that maternal VD_3 supplementation can protect the phospholipid content against lipid peroxidation of pork batters in offspring pigs, and improved the color stability [268]. Similar results were observed by previous researcher [201], who found that on storage days 7, dietary 25-OH-D_3 supplementation may maintain the color stability of muscle samples with an increased redness by delaying the lipid peroxidation of muscle samples. Meanwhile, the value of L^* and b^* of pork batters in offspring pigs from HD group were lower than those from LD group at 0, 24 and 48 h postmortem cold storage, which indicated that maternal VD_3 status inhibit lightness and yellowness of pork batters in offspring pigs and prolong the postmortem cold storage time.

Similar results were observed by a previous report [202], that found dietary VD_3 supplementation (125,000 μg) at 7 and 14 days postmortem decreased L^* values of pigs compared with the control group. Another study also observed that VD_3 supplementation (2000 μg) lowered L^* values of pork [200]. However, VD_3 supplementation did not affect b^* values of pork [200, 202]. Inconsistent research results might be due to the species or age, dosage of VD_3, and duration of feeding VD_3 supplementation. In addition, offspring pigs from HD group had no differences in L^* and a^* values at 0, 24 and 48 h postmortem storage, which indicated that maternal VD_3 supplementation in high dose inhibited the decrease of L^* and a^* values, and prolonged retail display of pork batters in offspring pigs during postmortem cold storage.

The texture of meat is usually considered to be the most important quality attributes that affecting consumer acceptance [269, 270]. The hardness, springiness, cohesiveness and chewiness of pork batters was increased by animal fat and sesame oil [271]. In the present study, the hardness, springiness, cohesiveness, and chewiness of pork batters of HD group were higher than that of LD group during all postmortem cold storage times, which suggested that maternal VD_3 supplementation in high dose improved texture properties of pork batters in offspring pigs. These results may be due to the increase of IMF content in offspring pigs by maternal VD_3 supplementation in high dose [21].

The underlying mechanism still need to be proven by further investigation. In addition, our study found that the hardness, chewiness, springiness and cohesiveness of pork batters in offspring pig from all groups decreased with postmortem cold storage time. Similar results also were observed [265], who found that textural properties of beef decreased with storage time, which was supported by the decrease in WHC. These results suggested that TPA parameters of pork batters were influenced by cold storage time. The reason may be that the cooking loss of pork batters increases with storage time (as shown in Table 1), and finally decreased the TPA parameters. Previous studies also found similar trends in sheep and camel muscle [272, 273]. The decrease of textural properties may be also caused by the degradation of porcine muscle by microorganisms and the endogenous enzyme [265]. However, other study observed that the TPA parameters of beef meat increased during storage period from 1 to 10 d [274]. Previous study also found that the hardness of porcine *longissimus dorsi* muscle was increased during chill storage [275]. The reason may be caused by the development of protein oxidation products in muscle samples during cold storage [276]. Inconsistent results in TPA parameters might be due to species, the temperature, and times of cold storage.

LF-NMR T_2 relaxation time could be used to characterize water distribution and fluidity in meat, which was helpful to understand the effects of autopsy, chilling and other factors on WHC in meat [218]. Previous study has demonstrated that T_{2a}, T_{21} and T_{22} represents the binding water combined with protein, water in the myofibrillar network of muscle, and free water, respectively [253]. In this study, there was no differences in T_{2a} of pork batters of offspring pigs were measured during cold storage in all-experimental groups, which indicated that binding water of pork batters of offspring pigs was not affected by maternal VD_3 status during cold storage period from 0 to 48 h postmortem.

In addition, HD group had lower T_{21} and T_{22} of pork batters than LD group at 0, 24 and 48 h postmortem cold storage. These results suggested that maternal VD_3 supplementation in high dose could inhabit water mobility, increase juiciness, and improve the quality of pork batters in offspring pigs during cold storage period. Our research also observed that offspring pigs born to LD, ND and HD groups had higher

T_{22} of pork batters at 48 h than those at 0 and 24 h postmortem cold storage, which indicated that water mobility was affected by postmortem cold storage.

4.4 Conclusions in section 4

1. Maternal VD_3 status affected pH value, WHC, shear force, meat color and LF-NMR relaxation times in offspring pigs during postmortem storage, which changed meat quality attribute and eating quality through regulating meat quality index.

2. Maternal high-dose VD_3 (3200 IU/kg basal diet) supplementation could improve meat quality function and technological properties, and prolong the freezing storage time of pork of offspring.

3. Maternal high-dose VD_3 status and cold storage time influenced cooking loss, meat color, texture properties, and low-field NMR T_2 relaxation time of pork batters in offspring pigs.

SECTION 5 RECOMMENDATIONS FOR IMPROVING THE TECHNOLOGY OF MEAT PRODUCTION WITH GIVEN FUNCTIONAL AND TECHNOLOGICAL PROPERTIES

This experiment systematically analyzed the influence of different maternal VD_3 levels during pregnancy on the meat quality characteristics, processing performance and technological properties of the offspring pork, thus clarifying the formation of functional and technical properties of pork by the influence of vitamin D_3 on the different maternal level of pigs. The experimental results will provide a theoretical basis and implementation plan for scientific production of high-quality pork products, improvement of pork quality functions, and improvement of meat quality technical properties. In addition, it can also provide the following suggestions for improving meat production technology with given functions and technical characteristics:

1. The 41st day gestation sow is the key window period for regulating the quality function and technical characteristics of offspring pork by means of nutrition (VD_3). Therefore, it is possible to produce high-quality offspring pork products by appropriately changing the nutritional level of VD_3 in the sows' diets at this time point.

2. From the 41st day of pregnancy to the farrowing period of sows, adjusting the level of VD_3 supplementation to 3200 IU/kg basic diet can significantly improve the quality characteristics and production performance of offspring pork. This experiment screened out the best VD_3 addition level of sows, providing a scientific feeding implementation plan for high-quality pork production.

3. Maternal high-dose VD_3 supplementation (3200 IU/kg basic diet) increased meat quality function and technology properties in pork and meat batters in offspring pigs during cold storage. So, maternal high-dose VD_3 supplementation could prolong the cold storage period and shelf life of pork and pork batters in offspring pigs.

4. Maternal high-dose VD_3 (3200 IU/kg basal diet) supplementation could also improve meat quality function and technological properties, and prolong the freezing storage time of pork of offspring.

Technological scheme and process of maternal VD_3 affecting pork quality of offspring pigs as shown in figure 5-1.

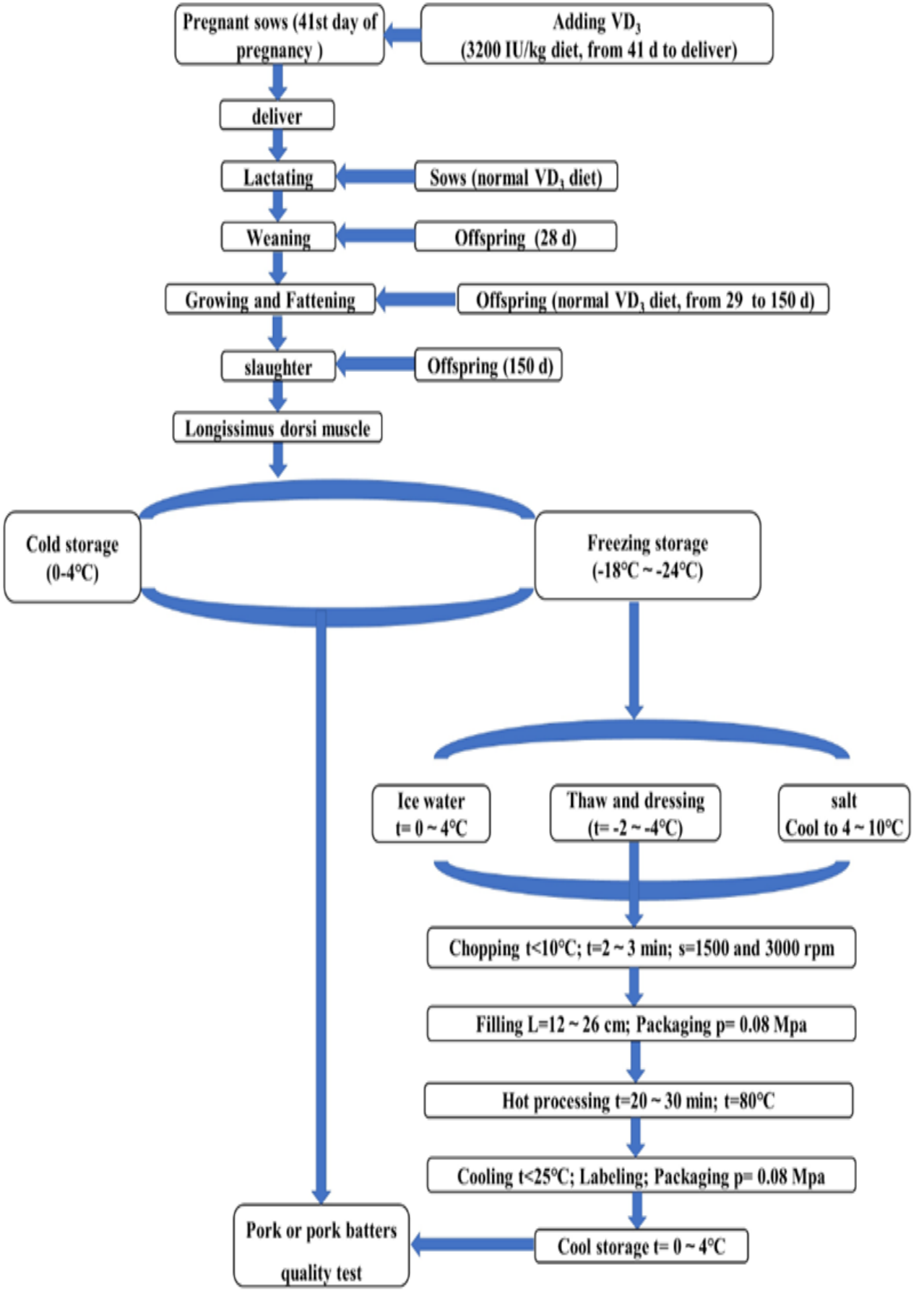


Figure 5-1 Technological scheme and technological process of Maternal VD3 affecting Pork quality of offspring pigs

First, it was determined that the VD_3 addition level of pregnant sows was 3200 IU/kg basic diet, and the feeding period was from the 41st day of gestation to the 114th day of gestation). After the piglets are delivered, the VD_3 level in the sows' diet shall be fed according to the recommended amount of NRC standard, and the piglets are lactating for 28 days. The piglets are weaned at 28 days of age, and then enter the growth and fattening period from 29 to 150 days of age.

When the piglets reach 150 days of age, slaughter them, collect the *longissimus dorsi* muscle and analyze the changes of meat quality function under cold storage and freezing storage conditions, so as to determine the technology of improving the function of refrigerated meat by VD_3 level of pregnant sows.

Finally, through the preparation of meat batters after thawing of frozen pork, it is used to determine the influence of maternal VD_3 level on the meat (batters) quality function and technological properties in offspring.

In addition, the conditions for preparing meat batters from frozen pork were determined, and the specific operations were as follows: After freezing, take out the pork from the refrigerator, put the pork and 2% sodium chloride into the meat grinder, and add ice water of 20% of the total amount of meat samples in three times. Chop for 30 seconds at 1500 r/min and repeat twice. After that, it was chopped at the speed of 3000 r/min for 60 seconds (the center temperature was lower than 10℃). After processing, take out the pork batters, put it into the packaging bag, boil it in 80 ℃ water bath for 20-30 min (the center temperature is 72 ℃), take it out, cool it at room temperature, and then store it in a 0-4 ℃ refrigerator.

CONCLUSIONS

The study was to conducted to investigated the formation of functional and technological properties of pork by the influence of Vitamin D3 on the differential maternal level of pigs.

The overall test results are as follows:

1. High-dose VD_3 maternal feeding can improve the pork quality function and technological properties of offspring by regulating key genes and serum hormones related to adipose tissue deposition.
2. Maternal VD_3 status during pregnancy have long-lasting impact on meat quality function and technological properties in offspring pigs.
3. Maternal VD_3 supplementation have positive effects on healthfulness of fatty acid profiles of offspring pork.
4. By adjusting the maternal VD_3 level during pregnancy, the pork quality characteristics of offspring were improved, and the scientific feeding scheme of VD_3 in early pregnancy sows was optimized.
5. Maternal VD_3 status affected pH value, WHC, shear force, meat color and LF-NMR relaxation times in offspring pigs during postmortem storage, which changed meat quality attribute and eating quality through regulating meat quality index.
6. Maternal high-dose VD_3 (3200 IU/kg basal diet) supplementation could improve meat quality function and technological properties, and prolong the freezing storage time of pork of offspring.
7. Maternal high-dose VD_3 status and cold storage time influenced cooking loss, meat color, texture properties, and low-field NMR T_2 relaxation time of pork batters in offspring pigs.

These results will provide a scientific basis for the early nutritional regulation of pork quality and process-ability (by regulating the level of VD_3 in pregnant sows), and maternal high-dose VD_3 (32000 IU/kg basal diet) during pregnancy (41^{st} day of pregnancy) could improve meat quality and processing performance in offspring pigs by altering lipid metabolism.

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APPENDICES

Supplemental table 1 Analyzed dietary VD_3 levels (IU/kg)[1]

Item	Gestation			Lactation	Offspring	
	LD	ND	HD		28-90 d	91-150 d
Formulated	200	800	3200	800	1215	1215
Analyzed	202	803	3210	802	1218	1219

[1] Dietary VD_3 levels were analyzed by using a combination of HPLC and mass spectrometry.

Supplemental table 2 Feed intake for sows and offspring pigs

Item	Treatment			S.E.M.	*P*-value
	LD	ND	HD		
Gestation sows (d 41 to birth)					
ADFI, kg	3.43	3.47	3.49	0.151	0.198
Lactation sows (d 0 to d 28)					
ADFI, kg	5.01	5.05	5.08	0.410	0.323
Lactation piglets (d 0 to d 28, weaning)					
ADFI, g	183.98	185.97	191.02	7.610	0.448
Growing piglets (d 28 to d 150)					
ADFI, kg	2.31	2.35	2.34	0.110	0.122

SEM, Standard error of the mean. ADFI, average daily feed intake.

Application testifies

By studying the effects of different VD_3 levels during pregnancy on lipid metabolism, meat quality characteristics and processing performance of offspring pigs, we determined that changing the VD_3 level of pregnant sows can effectively improve the quality characteristics and processing performance of offspring pork, and screened the best VD_3 addition level of pregnant sows (3200 IU/kg basic diet). The results will provide a scientific theoretical basis for the early nutritional regulation of pork quality during fetal period by regulating maternal dietary VD_3 status.

Our research results have been applied in Chinese pig breeding enterprises, such as Ji'an Nonghao Agricultural Development Co., Ltd, Henan heiyuan agriculture and animal husbandry technology Co., Ltd, and Chunfa farm in Shenqiu County. The application of the technology in these enterprises has obviously improved growth performance, meat quality of pigs, and the economic benefits of enterprises.

Application Testify

Application Enterprise	Ji'an Nonghao Agricultural Development Co., Ltd
Postal address	Chengxi Industrial Park, Jishui County, Ji'an City, Jiangxi Province, China
Starting and ending time	Sep, 6, 2020-now
Contact Person	Sun Shumeng (+86 17770662977)

Our enterprise has utilized the technology of Nutritional regulation of vitamin D_3 in pregnant sows to improve the quality of offspring pork. The technology can significantly regulate intramuscular fat content (from 1.62% to 2.54%), water holding capacity (from 75.58% to 79.62%) in offspring. Meanwhile, it can also improve meat color, shear force and meat quality attributes in offspring pigs. In our enterprise, the technology of adjusting maternal vitamin D_3 level during pregnancy to improve the quality characteristics of offspring pork has been widely used.

Ji'an Nonghao Agricultural Development Co., Ltd

Application Testify

Application Enterprise	Henan heiyuan agriculture and animal husbandry technology Co., Ltd
Postal address	No. 26, Jingwu Road, Jinshui District, Zhengzhou City, Henan Province, China
Starting and ending time	Nov,12, 2020-now
Contact Person	Liu Quanzheng (+86 18638799699)

Our enterprise has utilized the technology of Nutritional regulation of vitamin D_3 in pregnant sows to improve the quality of offspring pork from Nov 12, 2020 to now. The technology can significantly improve the growth performance, meat quality characteristics (intramuscular fat content, meat color, shear force, water holding capacity, fatty acid composition), eating quality and meat storage time in offspring pigs. The application of this technology in the company has significantly improved the economic benefits of the company of our company.

Henan heiyuan agriculture and animal husbandry technology Co., Ltd

Application Testify

Application Enterprise	Chunfa farm in Shenqiu County
Postal address	Yuanzhuang administrative village, Baiji Town, Shenqiu County, Henan Province
Starting and ending time	Nov, 25, 2020-now
Contact Person	Yang Zhigang (+86 15294774629)

Our farm has utilized the technology of maternal vitamin D_3 levels during pregnancy to improve the growth performance, meat quality of offspring from Nov 25, 2020 to now. The technology increased the growth performance, improved intramuscular fat content, meat color, shear force, fatty acid composition of longissimus muscle, and meat quality in offspring. The application of this technology in our farm has significantly improved the economic benefits by about 10%.

Chunfa farm in Shenqiu County