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EFFECTS OF MATERNAL VITAMIN D_3 LEVELS DURING PREGNANCY ON THE QUALITY CHARACTERISTICS OF PORK OF PIG OFFSPRING

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Abstract. Vitamin D_3 is considered an important nutritional regulatory factor of adipogenesis. It can regulate the quality characteristics of the meat of animals by affecting lipid metabolism. Maternal vitamin D_3 levels have long-lasting consequences for the meat quality and growth performance. This study has been conducted to investigate the effect that the maternal vitamin D_3 status in sows during pregnancy has on the meat quality attributes, chemical composition, and relaxation times of low field nuclear magnetic resonance (LF-NMR) T_2 of the longissimus dorsi muscle in pig offspring. **Materials and methods**. 27 sows (41^{st} day of pregnancy) were randomly divided into the low (LD), normal (ND), and high (HD) dietary vitamin D_3 groups (characterised by, respectively, 200, 800, and 3200 IU of vitamin D_3/kg experimental diet). In each group, there were 3 replicates with 3 sows per replicate. These diets were maintained throughout the sows' pregnancy up until parturition. From parturition to weaning, all lactating sows were fed according to the ND diet, and all piglets only obtained nutrition through breast milk without adding feed during the lactation period. At the age of 150 days, six offspring pigs in each group (2 offspring pigs with similar body weight per replicate, gender balanced) were selected and slaughtered to evaluate the pork quality characteristics. **Results and discussion**. The results have shown that maternal vitamin D_3 supplementation decreased the L*, b* value, shear force, cooking loss, T_{21} and T_{22} relaxation times, and increased the a* value, pH, water holding capacity (WHC), crude protein (CP), crude fat (CF), calcium (Ca), and phosphorus (P) content in the longissimus dorsi muscle of the pig offspring. These results demonstrated that a high-dose maternal vitamin D_3 level can improve the meat quality characteristics of pig offspring, and will provide a scientific basis for the early nutritional regulation of the quality of pork from offspring. **Conclusion.** The findings have indicated that high-dose maternal vitamin D_3 supplementation (3200 IU of vitamin D_3/kg experimental diet) has positive effects on the quality characteristics of the longissimus dorsi muscle and improves the eating quality of meat in pig offspring.

Keywords: Vitamin D_3, Pig offspring, Longissimus dorsi muscle, Meat quality, Chemical composition.

Introduction. Formulation of the problem

Pork is one of the most consumed red meat types in China, and its quality is closely associated with human health [1]. Growing evidence demonstrates that fat deposition in adipose tissue or muscle affects the nutritional value of meat. The suitable amount of intramuscular fat (IMF) can improve the taste, palatability, juiciness, tenderness, and flavour of meat, thereby enhancing meat quality in animals [2,3], and the IMF content positively correlates with the tenderness of meat [4,5].

Analysis of recent research and publications

Previous studies showed that the pork quality was usually influenced by the flavour, juiciness, and tenderness [6,7]. Besides, the juiciness and flavour of meat was also affected by the IMF content [8]. These results indicated that IMF affected meat quality characteristics, such as juiciness, tenderness, and flavour of pork [9]. The content of IMF in pigs mainly depends on the number of preadipocytes and the capacity of fat synthesis and accumulation [1], and is affected by many factors, which include nutrition, genetic background, and feeding methods. In recent years, the role of vitamin D_3 in fat synthesis has gradually attracted researchers' attention. More and more evidence showed that vitamin D_3 played an important regulatory role in the process of animal adipocyte anabolism [10,11].

A recent study showed that vitamin D was a nutritional regulator of adipogenesis and participated in regulation of lipid metabolism and fat accumulation by adjusting adipogenic gene expression [12]. Maternal vitamin D levels during pregnancy have a long-lasting effect on adiposity in offspring [13]. Maternal vitamin D deficiency can promote the differentiation and proliferation of preadipocytes [14]. Our previous study also observed that maternal vitamin D_3 supplementation during pregnancy improved the intramuscular fat (IMF) content in thelongissimus dorsi muscle of pig offspring [15]. These results revealed that maternal nutrition during pregnancy induced changes in adipogenesis, lipid metabolism, and meat quality [16,17]. Besides, maternal nutrient restriction or overnutrition also influenced the characteristics of the skeletal muscles, intramuscular triglyceride content of muscles, and fat accumulation in the offspring, which suggested that the early to mid-gestation period was very important for the development of skeletal muscles [18]. On the whole, the maternal vitamin D_3 status during pregnancy influences skeletal muscle development and adipogenesis of the offspring. Although vitamin D_3 is an important regulator of adipogenesis, little is clear about how maternal vitamin D_3 levels during pregnancy impact the pork quality in offspring of pigs.

Therefore, the purpose of this study was to evaluate the effects of maternal vitamin D_3 levels during pregnancy on the pork quality (colour, pH, shear force, cooking loss, and water holding capacity), chemical composition, and water distribution in thelongissimus muscle ofpig offspring.

Research objectives:

1. Analysing the effects of maternal vitamin D_3 on themeat quality attributes in the offspring.
2. Investigating the effect of maternal vitamin D_3 on the chemical composition of thelongissiums dorsi muscle in the offspring.
3. Evaluating the effect of maternal vitamin D_3 on LF-NMR relaxation times in thelongissiums dorsimuscle of the offspring

Research materials and methods

Animals and diets

The Animal Care and Use Committee of Henan University of Science and Technology approved all animals handling protocols in this study (Xinxiang, P.R. China). In this study, all animals and their diets used are the same as they werein our previous reports [19,20]. Seventy-two offspring piglets with similar body weights from 27 sows fed 200 (low vitamin D_3, LD), 800 (normal vitamin D_3, ND), and 3200 (high vitamin D_3, HD) IU of vitamin D_3/kg respectively were divided into 3 groups based on different levels of vitamin D_3fed to their mothers. Each group included 3 replicates with 8 offspring piglets per replicate. All lactating sows were fed the ND diet from delivery to weaning, and then all their offspring piglets were fed on the diet with the same vitamin D_3 levels. All experimental diets (Supplemental Table 1, Supplemental Table 2, and Supplemental Table 3) were formulated according to the recommendations of NRC (2012) [21]. The feeding trial lasted for 150 days, and all offspring pigs were kept under the same conditions.

Table 1 – Gestation diet composition of a sow[1]

Ingredient, %		Nutrients[3]	
Maize	61.91	DE, MJ/kg	13.03
Wheat bran	16	CP, %	16.45
Soya bean	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 diets for the low vitamin D_3 (LD), normal vitamin D_3 (ND), and high vitamin D_3 (HD) groups from 41 days of pregnancy until parturition. Their compositions were similar except for the vitamin D_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 vitamin D_3 (LD group), 800 IU of vitamin D_3 (ND group), 3200 IU of vitamin D_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 analysed values except for digestible energy, which was calculated using National Research Council values for swine (NRC).

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

Table 2 – Lactation diet composition of a sow[1]

Ingredient %		Nutrients[3]	
Maize	68	DE, MJ/kg	13.42
Wheat bran	8.02	CP, %	16.77
Soya bean	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 vitamin D_3 levels were fed to lactating sows in the low vitamin D_3 (LD), normal vitamin D_3 (ND), and high vitamin D_3 (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 vitamin D_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 analysed values except digestible energy, which was calculated using swine National Research Council values (NRC).

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

Table 3 –Ingredients and nutrients of the basal experiment diets of pig offspring

Item	28–90 days	91–150 days
Ingredient, %		
Maize	71.95	76.5
Soya bean	24	20
Limestone	0.7	0.9
$CaHPO_4$	1.7	1.2
Lysine	0.25	0.21
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 vitamin D_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 analysed values except digestible energy, which was calculated using swine National Research Council values (NRC).
DE, digestible energy. CP, crude protein. Ca, calcium. Lys, lysine. Met, methionine. Cys, cystine.

Slaughter and carcass measurements

At the end of the experiment, six offspring pigs in each group (2 offspring pigs with similar body weights per replicate, sex balanced) were selected to be slaughtered according to the previously reported method [9]. After slaughtering, the samples of the longissimus dorsi muscle in the offspring were quickly dissected and stored at 4℃ until analysis.

Analysis of the meat quality indicators

Colour measurement

After storage in a refrigerator at 4℃ for 24 h, the colour of the muscle samples of the pig offspring was measured with colorimeter (Konica Minolta CR 410, Sensing Inc., Osaka, Japan). The meat colour contained the L* (lightness), a* (redness), and b* (yellowness) values respectively [22].

PH value

The pH value of meat was determined in 24 h post mortem according to the methods described in the previous report [23]. Briefly, approximately 5 g of the longissimus dorsi muscle of each offspring pig was added into 45 ml of distilled water, mixed, and homogenised. After that, the pH value was measured with a pH meter (Model PC 510, Cyber scan, Singapore). The measurement of each sample was performed in triplicate, and its average value was calculated.

Shear force

Shear force in the muscle samples was determined using a digital explicit muscle tenderness meter CLM-4 (School of Engineering, Northeast Agricultural University). The shear force of raw meat was defined as the arithmetical mean of 10-cylinder maximum forces [24].

Cooking loss

The samples of the longissimus dorsa were separately packed in a plastic bag, and kept in water at 85℃ for 20 min. the samples of cooked meat were cooled to room temperature for 30 min. The surface exudate of the meat samples was wiped out with absorbent paper. The cooking loss of the meat samples was calculated based on the percentage weight ratio of the samples before and after cooking [23]. The equation is as follows:

$$\text{Cooking loss (\%)} = (M1-M2)/M1$$

In this equation, M1 and M2 indicate the weight of the longissimus dorsi muscle before and after cooking [25].

Water holding capacity

Water holding capacity (WHC) measurements were carried out according to the methods described in the previous reports [23,26]. Briefly, approximately 1 g of meat from each sample was added into a tube and centrifuged for 15 min (1500 g, 4℃). Then, a meat sample in the tube was taken out and weighed. All tubes were dried in an oven (at 105℃ for 24 h), and the WHC was calculated according to the method given in the previous report [4].

Chemical composition analysis

The dry matter (DM), crude protein (CP), crude fat (CF), calcium (Ca), and phosphorus (P) content of the longissimus dorsi muscle of the offspring were calculated according to the AOAC (Association of Official Analytical Chemists, 1990) procedures [27].

Low field nuclear magnetic resonance (LF-NMR)

The transverse relaxation time was measured as described in the previous reports [25,28], using an LF-NMR imaging analyser (NMI20-040V-I, Suzhou Newmai Analytical instrument Co., Ltd). The meat samples of the pig offspring were trimmed into cylinders of 1.5 cm in diameter and 3 cm long. They were placed in 5 cm high cylindrical tubes with the diameter 18 mm. The transverse relaxation time (T_2) was determined by the Carr-Purcell-Meiboom-Gill pulse sequence at 32℃, 200 μs (between 90° and 180° pulse), at the resonance frequency 22.4 MHz. Each pork sample (containing 8 scans) was analysed at a 3 s interval with 2000 echoes. Each meat sample was measured 3 times in parallel, and the average value was calculated.

Statistical analysis

ANOVA (statistical analysis of variance) was carried out using the procedure of one-way ANOVA in SPSS 17.0 for Windows. All treatment means were viewed as significant differences at $P<0.05$ by Duncan's multiple range tests. All data in this study were presented as mean±SEM.

Results of the research and their discussion

Meat quality indicators

As shown in Fig. 1, the pig offspring from the LD group had a lower a* value, but higher L* and b* values compared with those from the ND and HD groups (P<0.05). The WHC and pH values of the longissimus dorsi muscle of the pig offspring from the LD group were lower, while cooking loss was higher than that in the HD group (P<0.05). The shear force of the longissimus muscle of the ND group was higher than that of the HD group and lower than that of the LD group respectively (P<0.05).

The meat colour is considered the primary and direct determinant of consumers' purchase decision, and is used to assess the meat quality and freshness [29,30]. In this study, maternal vitamin D3 deficiency affected the red colour of pork (a*) and the sensory quality indicators of the pig offspring. These results suggested that the offspring pigs born to the LD group had worse meat quality. On the other hand, the LD group had higher L* and b* values compared with the HD and ND groups, which indicated that meat of the pigs born to the LD group were brighter and had worse meat quality characteristics. The longissimus dorsi muscle of the offspring from the HD group had higher WHC and pH values and lower cooking loss. These results may be due to the fact that a high pH value could improve the WHC and decrease the cooking loss [31]. Besides, the HD group had lower shear force compared with the ND and LD groups. This result may be due to the fact that adding a high dose of maternal vitamin D_3 improved the IMF content of the longissimus dorsi muscles in the offspring by enhancing the FAS expression and FAS/HSL ratio, which led to decrease in the shear force of the pork [15]. The previous study also found that the IMF content positively correlated with the tenderness of meat [32]. Poor WHC can lead to higher drip loss, and thus to loss in the carcass weight and a decrease in the meat quality [33]. In our study, the pig offspring born to the LD group had lower WHC than those born to the HD group, which revealed that maternal vitamin D_3 supplementation in low doses (deficiency) decreased the yield and quality of meat in the pig offspring.

Chemical composition analysis

As shown in Table 4, the CP, CF, and P levels of the longissimus dorsi muscle of the pig offspring from the HD group was higher than of those from the LD group (*P*<0.05), whereas no differences were observed between the ND and LD groups, or the ND and HD groups (*P*>0.05). The Ca content of the longissimus dorsi muscle of the ND group was higher than that of the LD group and lower than that of the HD group respectively (*P*<0.05). However, no difference in the DM content was observed among all experimental groups (LD, ND, and HD groups, *P*>0.05).

The offspring pigs born to the LD and ND groups had lower CP, CF, Ca, and P contents of the longissimus dorsi muscle compared with those born to the HD group. These results indicate that maternal high-dose vitamin D3 can increase the lean meat yield and IMF content of the longissimus dorsi muscle of pig offspring. Similar results were observed in the previous report, which found that high administration of maternal vitamin D3 increased the muscle fibre number of the longissimus dorsi muscle of the offspring piglets [34].

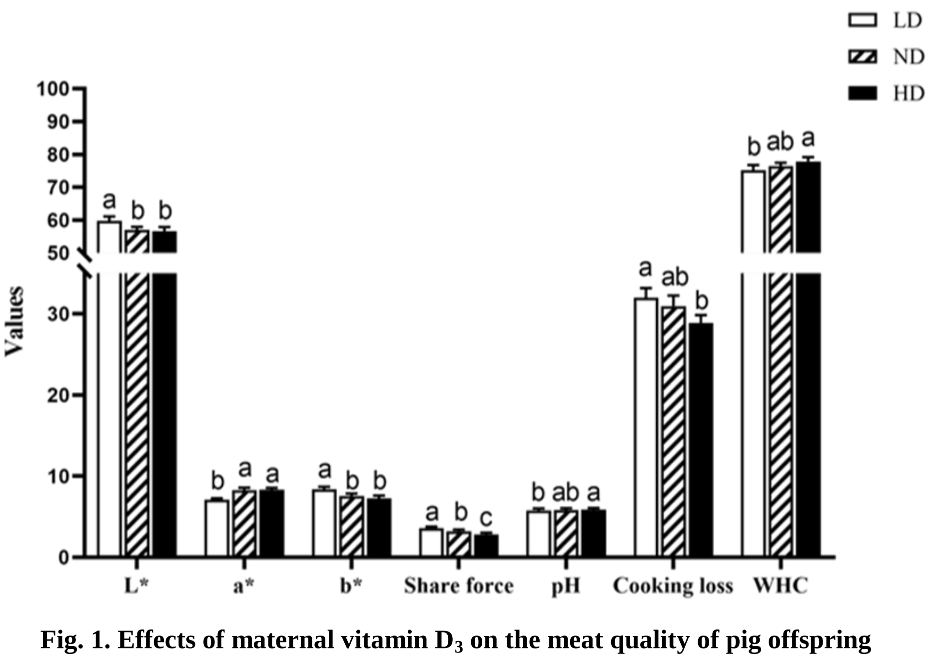

Fig. 1. Effects of maternal vitamin D_3 on the meat quality of pig offspring

a, b, c = bars with different superscripts for each characteristic of the meat quality separately are significantly different at *P*<0.05. LD, low vitamin D_3 group. ND, normal vitamin D_3 group. HD, high vitamin D_3 group.
L^*, lightness. a^*, redness. b^*, yellowness. WHC, water holding capacity.

Table 4– Effects of maternal vitamin D_3 on the chemical composition of the longissimus dorsi muscles in the offspring of pigs

Item	Groups			SEM	*P*-value
	LD	ND	HD		
DM, %	33.41[a]	33.73[a]	34.12[a]	2.359	0.445
CP, %	18.84[b]	19.65[ab]	20.88[a]	0.521	0.031
CF, %	9.21[b]	10.42[ab]	12.31[a]	0.522	0.022
Ca, %	0.26[c]	0.37[b]	0.44[a]	0.033	0.001
P, %	0.11[b]	0.18[ab]	0.24[a]	0.011	0.001

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

LD, low vitamin D_3 group. ND, normal vitamin D_3 group. HD, high vitamin D_3 group. DM, dry matter. CP, crude protein. CF, crude fat. Ca, calcium. P, phosphorus.

Our previous study also observed that maternal vitamin D_3 supplementation increased the IMF content of the longissimus dorsi muscle of the offspring piglets [15]. Previous results also made it clear that vitamin A administration at birth promoted the calf growth and IMF development in beef cattle [35]. These results suggested that maternal nutrients could affect the CP or IMF content of the longissimus dorsi muscle and improve the chemical composition and meat quality indicators of thepig offspring. Vitamin D is also an essential nutrient for maintaining the calcium homoeostasis and bone metabolism, which is mainly responsible for the intestinal absorption of phosphate, calcium, and magnesium [36]. In this study, the offspring pigs born to the HD group had higher Ca and P contents of the longissimus dorsi muscle compared with those born to the LD group, which revealed that the maternal vitamin D_3 status during pregnancy could influence Ca and P concentrations of the longissimus dorsi muscle in the pig offspring. Taken together, the results have indicated that the maternal vitamin D3 status during pregnancy can improve the chemical composition of the muscle and the meat quality of pig offspring.

LF-NMR. As shown in Fig. 2, there are no differences in the T2a relaxation times in the muscle samples of the offspring from the LD, ND, and HD groups (P>0.05). The T21 relaxation time of longissimus dorsi muscles in the HD group was lower than that in the LD group (P<0.05). The offspring pigs from the ND and HD groups had lower T22 relaxation times compared with those from the LD group (P<0.05).

The pig offspring decreased with the increase of maternal vitamin D_3 supplementation. These results suggest that maternal vitamin D_3 deficiency resulted in immobile water changing to free water and decreased water abundance in the longissimus dorsi muscles in the pig offspring. T_{22} of the longissimus dorsi muscle in the HD group was lower compared with the LD group. The result revealed that the maternal vitamin D_3 levels during pregnancy affected the mobility of water in the offspring pigs 'longissimus dorsi muscle. Compared with the LD group, the pig offspring born to the HD group had lower water mobility, which reflected higher juiciness and WHC of the longissimus dorsi muscle. Previous reports showed that tenderness correlated with T_2 and was also closely associated with the amount of moisture [42]. These findings suggest that the WHC and meat quality of the offspring was influenced by the maternal vitamin D_3 levels during pregnancy. Besides, maternal vitamin D_3 deficiency could also decrease the tenderness of the longissimus dorsi muscles in the pig offspring. However, the mechanism needs to be evaluated by further investigation.

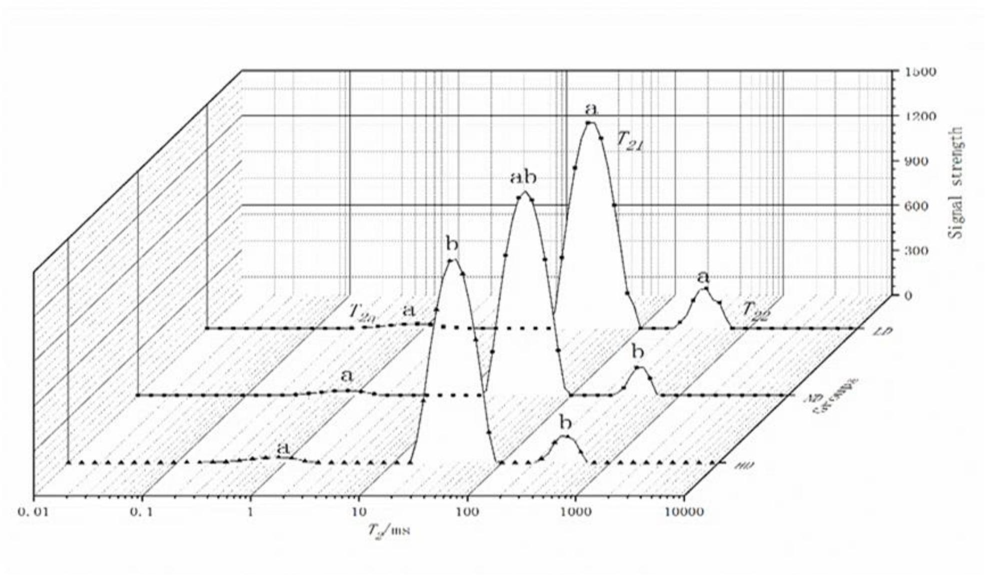

Fig. 2. Three-dimensional T2 relaxation time plot of longissimus muscles in the pig offspring from the LD, ND, and HD groups

T_{2a}, binding water relaxation time. T_{21}, immobile water relaxation time. T_{22}, free water relaxation time. LD, low vitamin D_3 group. ND, normal vitamin D_3 group. HD, high vitamin D_3 group.

Conclusion

High-dose supplementation of maternal vitamin D_3 decreased the L*, b* value, shear force, cooking loss, and T21 and T22 relaxation times, but increased the a* value, pH value, water holding capacity (WHC), crude protein (CP), crude fat (CF), calcium (Ca), and phosphorus (P) content in thelongissimusdorsimuscle of the pig offspring. These results indicate that highdoses ofmaternal vitamin D3 improved the eating quality of meat in the pig offspring by regulating the chemical composition, water distribution, and meat quality index of muscle.

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Declaration of competing interest. We declare that we have no conflict of interest.

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