Effects of maternal vitamin D$_3$ status on meat quality and fatty acids composition in offspring pigs

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ABSTRACT. Eighteen offspring pigs (150 days of age, sex balanced, similar body weights, 6 offspring per maternal diet group) from sows fed diets with different vitamin D$_3$ levels (200, 800 and 3200 IU/kg basal diet) were weighed and slaughtered to examine the influence of maternal vitamin D$_3$ levels on meat quality and fatty acids composition. The results suggested that maternal vitamin D$_3$ supplementation decreased drip loss, shear force, total saturated fatty acids (SFA) content and n-6:n-3 ratio, while increased marbling score, subjective colour score, longissimus muscle area, total monounsaturated fatty acids (MUFA) content, polyunsaturated fatty acids (PUFA) content and the PUFA:SFA ratio. It was revealed that maternal vitamin D$_3$ supplementation exerts positive effects on the meat quality of offspring pigs, and improves the healthful attributes of fatty acid profile. It can be concluded that an appropriate maternal vitamin D$_3$ level (3200 IU of vitamin D$_3$/kg basal diet) may improve the meat quality of offspring pigs.

Introduction

The content of intramuscular fat (IMF) is correlated with meat palatability (Hausman et al., 2009; Du et al., 2010a,b). Intramuscular adipocytes are generated at the foetal and neonatal stages (Tong et al., 2008), and they generate marbling at the fattening period in offspring. It was observed that maternal nutrition during pregnancy could induce changes in the physiology, metabolism and meat quality of offspring (Wu et al., 2004; Symonds et al., 2007). Maternal malnutrition or overnutrition affects the number of myofibres, fat accumulation and intramuscular triglyceride content of skeletal muscle in offspring, which demonstrates that the early to mid-gestation period is crucial for skeletal muscle development and fat adipogenesis (Zhu et al., 2006). Growing evidence found that vitamin D$_3$ plays a regulatory function in the foetal muscle development in animals (Endo et al., 2003; Hines et al., 2013; Flohr et al., 2016). Meanwhile, muscle growth potential in animals is positively correlated with the total number of muscle fibres at the foetal stage (Dwyer et al., 1993; 1994). These findings suggested that vitamin D$_3$ may affect postnatal muscle development and meat quality in animals by improving muscle fibre number during the foetal period. Additionally, vitamin D$_3$ is a regulatory factor in adipogenesis (Wang et al., 2016). It was found that maternal vitamin D$_3$-deficient offspring rats had higher proliferation and a greater number of lipid droplets in preadipocytes than the control ones (Wen et al., 2018). We have also found that maternal vitamin D$_3$ supplementation could affect adipogenic
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genes expression, intramuscular fat accumulation and pork quality in offspring pigs during frozen storage (Guo et al., 2020a,b,c; 2021). Therefore it is suggested that maternal vitamin D₃ levels during pregnancy affect lipid metabolism of offspring. In previous reports it was also observed that dietary vitamin D₃ level affects pork quality by regulating shear force, pH value and meat colour (Wilborn et al., 2004; Duffy et al., 2018). In other studies it was found that short-term feeding with vitamin D₃ can improve meat colour, but does not change the tenderness of pork-loin chops (Wiegand et al., 2002). Such results revealed that dietary vitamin D₃ supplementation could improve pork quality.

Although, vitamin D₃ plays a key role in the regulation of fat accumulation and lipid metabolism, still little is known about the effect of maternal vitamin D₃ on meat quality in offspring pigs. Therefore, the aim of the study was to assess the effect of maternal vitamin D₃ levels during pregnancy on the marbling score, subjective colour score, *longissimus* muscle area, shear force, drip loss and fatty acid composition in the *longissimus* muscle of offspring pigs.

### Material and methods

#### Animals and diets

All animals’ handling protocols were approved by the Animal Care and Use Committee of Henan Institute of Science and Technology (Xinxiang, P.R. China).

The animals and diets used in the present study were the same as in our previous reports (Guo et al., 2020a; 2021). Briefly, 27 pregnant sows with similar body weights (144.62 ± 2.13 kg, the same parities) were randomly allotted to low (LD), normal (ND) and high (HD) vitamin D₃ groups in which the sows’ diets were supplemented with 200, 800 and 3200 IU of vitamin D₃/ kg diet, respectively. Each group contained 3 replicates with 3 sows per replicate. The experimental sows were supplemented with different levels of vitamin D₃ from the 41st day of pregnancy to delivery. All sows were housed and fed individually. Vitamin D₃ (feed grade) was characterized by 500,000 IU per g and was provided by Zhejiang Garden Biochemical High-Tech Co., Ltd (Hangzhou, China).

The born piglets were used in the next part of the study according to the mothers’ diet. 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 piglets born by 27 sows were allotted into 3 groups (every group containing 3 replicates with 8 offspring per replicate) again according to the maternal vitamin D₃ supplementation group. Offspring pigs from all groups were fed diet with the same vitamin D₃ level, and were housed and fed under the same conditions. All diets were formulated according to the National Research Council (NRC, 2012) recommendations. Because the diets used for sows and offspring pigs in this study were the same as those in our previous studies, the experimental diets are shown in Supplemental Table 1, Supplemental Table 2 and Supplemental Table 3 available only online at www.jafs.com.pl.

### Slaughter and carcass measurements

At 150 day of age, 6 offspring pigs from each group (2 pigs per replicate, sex balanced, similar body weights) were selected and slaughtered according to the method previously reported by Miao et al. (2009). Briefly, the pigs were electrically stunned, exsanguinated, dehaired and eviscerated after 12 h of feed deprivation. The head was removed and the carcass was split longitudinally, the *longissimus* muscle was separated from the left half-carcasses after an overnight (12 h) chill at 4 °C. Then, *longissimus* muscle area 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 °C for 12 h, the shear force of the *longissimus* muscle was measured according to the method previously reported by Laville et al. (2007). The muscle sample was put into a water bath at 80 °C for 30 min until the central temperature reached approximately 72 °C, and then it was removed and cooled to a central temperature of about 4 °C 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 long), after discarding records that differ from the mean value of 10 records by more than 2 standard deviations. All the shear force analyses were conducted without freezing the meat in advance. The *longissimus* muscle samples were stored at 4 °C 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 colour was determined using colour standards (7-colour discs), which ranged from 1 (greyish-white) to 7 (deep red).
Marbling scores (National Pork Producers Council Standards, NPPC, 2000) were determined by colour standards (5-colour discs), which ranged from 1 (pale pinkish to grey) to 5 (dark purplish to red). The subjective colour and marbling scores were determined at approximately 48 h post-mortem.

**Fatty acids composition analysis**

For fatty acid analysis, intramuscular fat was extracted from *longissimus* muscle samples according to the method based on fatty acid methyl ester (FAME) synthesis previously reported (O’Fallon et al., 2007; Jin et al., 2018; Waheed et al., 2018). 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 °C in water for 1.5 h. After cooling in the cold water bath, 0.58 ml of H₂SO₄ was added to the tube. The tube was mixed and incubated at 50 °C in water for 1.5 h once 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 analysed on an Agilent Technologies 7890A gas chromatograph (GC; Model 7890A, Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-23 60 m × 0.25 mm capillary column with a 0.25-µm film thickness (Agilent Technologies, Palo Alto, CA, USA). The operating programme was as follows: injection volume of 1 µl, injector temperature of 250 °C, detector temperature of 300 °C, and initial column temperature of 140 °C for 5 min that was then raised to 220 °C at 5 °C per min and kept for 16 min. The fatty acids were determined by a flame ionization detector, chromatograms were analysed with the use of a gas chromatography ChemStation Software (Agilent Technologies, Palo Alto, CA, USA). Results were expressed as the percentage of the total fatty acids identified and grouped as follows: saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). In addition, the PUFA:SFA and n-6:n-3 ratios were calculated.

**Statistical analysis**

The one-way ANOVA procedure of SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for performing statistical analysis of variance (ANOVA). The model mainly examined the effects of maternal vitamin D₃ status during pregnancy on meat quality and fatty acids composition in offspring without considering sex comparison of offspring. Significant differences among all treatment means (offspring pigs) were determined by Duncan’s multiple range tests; significance was declared at *P* < 0.05. Replicates (*n* = 6) were used as treatment unit and analysed individually for the study of pork quality parameters (marbling score, subjective colour score, longissimus muscle area, drip loss and shear force) and fatty acids composition in offspring pigs. All data were presented as mean ± standard error of means (SEM).

**Results**

**Meat quality.** As shown in Table 1, offspring pigs from the LD group 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 colour score in offspring pigs born in HD group was higher than that in pigs born in LD and ND groups (*P* < 0.05). Additionally, *longissimus* muscle area of offspring from ND group 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* 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).

**Table 1. Effects of maternal vitamin D₃ supplementation on pork quality in offspring pigs**

| Indices                     | Groups¹ | SEM | *P* value |
|-----------------------------|---------|-----|-----------|
| Live weight, kg             | LD      | 94.89 | 92.72 | 94.27 | 0.95 | 0.224 |
| Marbling score              |         | 1.47ᵃ | 1.87ᵇ | 1.93ᶜ | 0.07 | 0.001 |
| Subjective colour score     |         | 2.71ᵃ | 2.83ᵇ | 3.19ᶜ | 0.10 | 0.004 |
| Longissimus muscle area, cm²|         | 32.42ᵃ | 33.60ᵇ | 35.39ᶜ | 0.43 | 0.002 |
| Drip loss, %                |         | 4.82ᵃ | 4.61ᵇ | 4.18ᶜ | 0.14 | 0.002 |
| Shear force, Newton         |         | 35.28ᵃ | 31.36ᵇ | 27.44ᶜ | 1.33 | 0.002 |

¹Groups according to maternal vitamin D₃ supplementation: LD – low vitamin D₃ group; ND – normal vitamin D₃ group; HD – high vitamin D₃ group; SEM – standard error of the means; ⁡⁺⁺ means in the same raw with different superscripts are significantly at *P* < 0.05

**Fatty acids composition.** As shown in Table 2, the offspring pigs born in LD group had higher total SFA content in the *longissimus* muscle samples than those born in HD group (*P* < 0.05), while no difference was found between LD and ND groups or between HD and ND groups (*P* > 0.05). The C14:0, C16:0 and C18:0 fatty acids concentrations of offspring pigs from ND and HD groups were significantly lower than in LD group (*P* < 0.05), and no difference was observed between ND and HD groups (*P* > 0.05). In addition, there was no difference in C20:0 concentrations among all groups (*P* > 0.05).
Table 2. Effects of maternal vitamin D3 supplementation on fatty acids composition of longissimus muscle in offspring pigs, % except for PUFA:SFA and n-6:n-3 ratios

| Indices | Groups | SEM | P-value |
|---------|--------|-----|---------|
|         | LD     | ND  | HD      |
| SFA     |        |     |         |
| C14:0   | 2.58a  | 2.22a| 2.13b  | 0.14 | 0.002 |
| C16:0   | 27.68a | 25.22a| 24.21b | 0.32 | 0.001 |
| C18:0   | 13.24a | 11.69a| 10.35b | 0.12 | 0.002 |
| C20:0   | 0.16   | 0.15 | 0.14    | 0.09 | 0.062 |
| MUFA    |        |     |         |
| C16:1   | 1.88a  | 2.24a| 2.46b  | 0.04 | 0.001 |
| C18:1n-9| 32.55a | 33.21a| 34.44a | 0.34 | 0.044 |
| C20:1   | 1.07a  | 1.14a| 1.16b  | 0.03 | 0.031 |
| PUFA    |        |     |         |
| C18:2n-6| 18.84a | 21.43a| 21.26b | 0.09 | 0.001 |
| C18:3n-3| 0.63a  | 0.84a| 1.06b  | 0.04 | 0.039 |
| C20:2n-6| 0.31a  | 0.38a| 0.46b  | 0.02 | 0.042 |
| C20:3n-3| 0.81a  | 0.94a| 1.14b  | 0.06 | 0.046 |
| C20:4n-6| 0.25a  | 0.34a| 0.39b  | 0.02 | 0.021 |
| Total SFA| 43.66a | 39.28a| 36.83a | 0.62 | 0.014 |
| Total MUFA| 35.50a | 36.59a| 38.06a | 0.26 | 0.003 |
| Total PUFA| 20.84a | 24.13a| 25.11a | 0.19 | 0.001 |
| PUFA:SFA ratio | 0.48a | 0.61a| 0.68b  | 0.02 | 0.002 |
| n-6:n-3 ratio | 13.47a | 12.55a| 10.41b | 0.16 | 0.001 |

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; Groups according to maternal vitamin D3 supplementation: LD – low vitamin D3 group, ND – normal vitamin D3 group, HD – high vitamin D3 group; SEM – standard error of the means; * – means in the same row with different superscripts are significantly at P < 0.05.

Total MUFA concentrations of longissimus muscle in offspring pigs from HD group were higher than those in pigs from LD group (P < 0.05), and no difference was observed between LD and ND groups, as well as between HD and ND groups (P > 0.05). Meanwhile, in offspring pigs from HD and ND groups higher concentrations of C16:1, C18:1 and C20:1 fatty acids than in LD group (P < 0.05) were noted.

The offspring pigs from ND and HD groups had higher total PUFA concentrations and PUFA:SFA ratio values than offspring pigs born in LD group (P < 0.05). Meanwhile, the levels of C18:2 and C20:4 fatty acids of offspring pigs from ND and HD groups were higher than in pigs from LD group (P < 0.05). The C18:3, C20:2 and C20:3 fatty acids levels in offspring pigs from HD group were higher, while n-6:n-3 ratio values were lower than in those from LD group (P < 0.05), while no difference was found between LD and ND groups, as well as between ND and HD groups (P > 0.05).

**Discussion**

In the presented study the effects of maternal vitamin D3 status on the meat quality of offspring pigs during cold storage have been investigated for the first time. It was shown that the marbling score and the subjective colour score of meat from offspring pigs were influenced by maternal vitamin D3 levels. Both marbling score and subjective colour score in offspring from HD group were higher than that from ND and LD groups. So it was revealed that maternal vitamin D3 supplementation improved meat quality in offspring. The reason may be that the high dosage of maternal vitamin D3 increases the intramuscular fat content in offspring pigs. In the present study, the offspring from HD group were characterised by better meat quality as they were of lower pale, drip loss, shear force, and had higher longissimus muscle area in comparison with ND and LD groups. Such results may suggest that meat quality in offspring pigs can be influenced by maternal vitamin D3 status during pregnancy. Similar results were also found in our previous study (Guo et al., 2020c), in which it was stated that maternal vitamin D3 supplementation improved meat quality in offspring pigs during frozen storage by inhibiting the decrease of cooking loss, drip loss and thawing loss of longissimus muscle. The present and previous results indicate that high-dose maternal vitamin D3 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 intramuscular fat content and fatty acids composition in animals (Fisher et al., 2000). The increase in the degree of lipid unsaturation in meat is beneficial for human health (Simopoulos, 2008), lower SFA and higher n-3 fatty acids concentrations improve meat quality and so reduce cardiovascular diseases risk (Hu et al., 2001). In this study, maternal vitamin D3 supplementation during pregnancy decreased total SFA and increased n-3 PUFA concentrations in offspring pigs, which indicated that maternal high-dose vitamin D3 levels regulated fatty acids composition of longissimus muscle in offspring. Meanwhile, the higher SFA content and n-6:n-3 ratio are associated with the pathogenesis of many diseases, such as diabetes, coronary heart disease, cancer, cerebrovascular and cardiovascular diseases in humans (Katan, 2000; Liu and Zhou, 2013). The present study has shown that the total SFA and n-6:n-3 ratio of longissimus muscle in offspring pigs were reduced by maternal vitamin
D₃ supplementation. It was indicated that maternal vitamin D₃ supplementation regulated meat quality by affecting the composition of fatty acids.

Conclusions
It was demonstrated that maternal vitamin D₃ supplementation at a high dose improved pork quality by increasing marbling score, subjective colour score and longissimus muscle area, and decreasing shear force and drip loss. Meanwhile, increased total polyunsaturated fatty acid (PUFA) and monounsaturated fatty acids (MUFA) concentrations, and decreased n-6:n-3 ratio in longissimus muscle were stated, which revealed that maternal high-dose vitamin D₃ addition regulated fatty acids composition of longissimus muscle in offspring pigs. So, maternal high-dose vitamin D₃ supplementation during pregnancy can affect the meat quality parameters and fatty acids composition of offspring pigs. Nevertheless the mechanism of vitamin D₃ action is still unclear and needs to be further explored.

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Conflict of interest
The authors declare that there is no conflict of interest.

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