



Effects of maternal vitamin D₃ on quality and water distribution in pork of offspring pigs during frozen storage

L. Guo^{1,3}, Z. Miao², S. Melnychuk^{1,4} and H. Ma³

¹ Sumy National Agrarian University, Faculty of Food Technology, H. Kondratieva st, 40021, Sumy, Ukraine
Henan Institute of Science and Technology

² College of Animal Science and Veterinary Medicine, ³ School of Food Science and Technology
Eastern HuaLan Avenue 453003, Xinxiang, China

KEY WORDS: maternal vitamin D₃,
longissimus dorsi muscle, meat quality,
water distribution, offspring pigs

Received: 28 September 2020

Revised: 20 November 2020

Accepted: 9 December 2020

⁴ Corresponding author:

e-mail: serge.melnichuk@gmail.com

ABSTRACT. In total, 27 sows (41st day of pregnancy) were divided into three groups: LD, ND and HD, and fed 200, 800 and 3200 IU of vitamin D₃/kg basal diet, respectively. All their offspring pigs were fed the same vitamin D₃ replete diet. On day 150, 18 offspring (sex balance, 6 offspring per maternal diet group) were weighed and slaughtered to investigate effects of maternal vitamin D₃ status on quality and water distribution in pork during frozen storage. It was shown that in *longissimus dorsi* muscles from all groups L^* , b^* values, thawing loss, cooking loss, T_{21} and T_{22} relaxation times increased with frozen storage ($P < 0.05$), whereas a^* value and shear force decreased with frozen storage ($P < 0.05$). In addition, in comparison with HD group, meat from LD group had higher L^* and b^* values, thawing loss, shear force, and T_{22} relaxation times ($P < 0.05$). Whereas cooking loss and T_{21} relaxation times of *longissimus dorsi* muscles in offspring born in HD group were lower ($P < 0.05$) than that in those born in ND and LD groups, while a^* values of meat from LD group were lower ($P < 0.05$) than that from ND and HD groups during frozen storage. It was indicated that maternal vitamin D₃ status influenced meat colour, thawing loss, cooking loss, shear force and water distribution of *longissimus dorsi* muscles in offspring, and maternal vitamin D₃ supplementation could decrease quality deterioration of muscles, and prolong meat quality during frozen storage.

Introduction

Vitamin D₃ is an important regulator of adipogenesis, and it is correlated with fat accumulation. It was found that maternal vitamin D₃ status influenced adipocyte commitment and differentiation of muscle *longissimus dorsi* in piglets via changing adipogenic genes expression (Guo et al., 2020a). Meanwhile, maternal vitamin D₃ affected intramuscular fat (IMF) content and meat quality of offspring pigs by regulating fatty acid synthesis and hormone sensitive lipase mRNA expression (Guo et al., 2020b). In addition, vitamin D also has important direct

effects on skeletal muscle (Abrams et al., 2018), and improving maternal vitamin D₃ status can promote prenatal skeletal muscle development in offspring pigs by regulating the expression of muscle transcription factors (Zhou et al., 2016). These results suggested that maternal vitamin D₃ concentrations affected meat quality attributes and eating quality of offspring pigs. It was previously found that fat accumulation, especially the suitable amount of IMF could significantly improve the taste of meat, palatability, juiciness, tenderness, flavour and, finally, the meat quality (Hunt et al., 2014; Bauer et al., 2016). The eating quality of pork is usually affected

by flavour, tenderness and juiciness (Aaslyng et al., 2003; Miao et al., 2016). Lipid oxidation and degradation, and the water-soluble components in meat (sugars and amino acids induced by Maillard reaction) has been considered to influence flavour deterioration in meat (Mottram, 1998). 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 (Miao et al., 2016), and there is a positive correlation between IMF content and meat tenderness (Houbak et al., 2008; Magnabosco et al., 2016). The juiciness of meat is often influenced by raw meat quality and cooking procedure, and it is correlated to the IMF content in pork. It was previously noted that higher content of IMF is associated with better meat quality (Fernandez et al., 1999; D'Souza and Mullan, 2002; Ventanas et al., 2007). Frozen storage is an important method for long-term preservation of pork quality, nutritional value, colour and flavour of meat, however it reduces juiciness, tenderness, as well as eating quality, nutritional value, colour and flavour of meat, reduces juiciness and tenderness, as well as decreases eating quality (Zhang et al., 2017a; Zhang et al., 2019). It was shown that ice crystals will disrupt muscle cells and cause quality loss in muscle tissue during frozen storage. Water plays an important role in meat production, and the distribution of water in meat affected juiciness and tenderness of meat (Trout, 1988). Freezing and thawing could increase drip loss, and decreases sensory quality of meat. Bound, immobilized and free water in muscle tissues are main types of water components. In addition, ice crystal in muscle tissues was formed by immobilized and free water during freezing (Xia et al., 2009). The changes in water mobility and distribution of meat can be investigated through low field nuclear magnetic resonance (LF-NMR) (Zhang et al., 2017a). Although, freezing could influence meat quality and prolong the shelf life of meat. However, there were little reports about effects of maternal vitamin D₃ on quality and water distribution in *longissimus dorsi* muscle of offspring piglets during frozen storage. Therefore, the present study was aimed to investigate the changes of thawing loss, colour, cooking loss and water distribution in *longissimus dorsi* muscle of offspring pigs during frozen storage. These data would provide the theory basis for the prolongation and protection of pork quality in offspring pigs during frozen storage by maternal vitamin D₃ nutrition. So, it was hypothesized that maternal vitamin D₃ supplementation would improve meat quality attributes and water holding capacity of *longissimus dorsi* muscles in offspring pigs during frozen storage.

Material and methods

Animals

In total, 27 pregnant sows (41st days of pregnancy) with the same parities and similar body weights (144.6 ± 2.3 kg) were randomly divided into low vitamin D₃ (LD), normal vitamin D₃ (ND) and high vitamin D₃ (HD) groups, each group includes 3 replicates with 3 sows per replicate, which were fed 200, 800 and 3200 IU of vitamin D₃/kg basal diet, respectively (Table 1). Animals were fed these diets throughout pregnancy until giving birth. From giving birth to weaning (day 28 of piglet age), all lactating sows were switched to the ND diets.

Table 1. Composition of gestation and lactation diets fed to sow

Indices	Gestation diet	Lactation diet
Ingredient, %		
maize	61.91	68
wheat bran	16	8.02
soyabean	19	20
fish meal	0	1
limestone	1.5	1.5
CaHPO ₄	0.29	0.18
salt	0.3	0.3
premix ¹	1	1
total	100	100
Nutrients ²		
digestible energy, MJ/kg	13.03	13.42
crude protein, %	16.45	16.77
Ca, %	0.68	0.70
available P, %	0.36	0.36
lysine (Lys), %	1.04	1.09
methionine (Met), %	0.24	0.27
Met+Cys, %	0.52	0.54
Vitamin D ₃ , IU/kg		
LD group	200	
ND group	800	800
HD group	3200	

¹ provided per kg of diet, mg: Cu 10 (lactation diet), 20 (gestation diet), Fe 80, Mn 25, Zn 100, I 0.2, Se 0.2, vit. K₃ 1, vit. B₁ 1, riboflavin 3.75, vit. B₆ 1, vitamin B₁₂ 15, pantothenic acid 12, niacin 10, choline 1.25; IU: vit. A 4000 (lactation diet), 2000 (gestation diet), vit. E 44;

² analysed values except digestible energy calculated according to swine National Research Council (NRC, 2012) values

During this period, all sows in gestation and lactation were housed in temperature-controlled gestation stalls (2.1 × 0.6 m) and farrowing crates (2.1 × 0.6 m for the sow and 2.1 × 1 m for the offspring pigs), respectively. After weaning, 72 offspring piglets (sex balance) from the group of 348 offspring were allotted into 3 groups depending on the level of the vitamin D₃ given to their mothers. Each group has 3 replicates with 8 offspring piglets (sex balance) per replicate. All offspring were fed the same vitamin

D₃ replete diets (Table 2), and reared in temperature-controlled pens from days 28 to 150 of age. At their pre-designated slaughter age (day 150 of age), 6 offspring pigs with similar body weight from each group (2 offspring pigs per replicate, sex balance) were randomly selected to weigh and slaughter for tissue collection. In this study, all sows and their offspring pigs had *ad libitum* access to an experimental diet, and water *via* nipple drinkers.

Table 2. Composition of diets fed to offspring pigs

Indices	28–90 days of age	91–150 days of age
Ingredient, %		
maize	71.95	76.5
soyabean	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		
digestible energy, MJ/kg	13.75	13.79
crude protein, %	17.78	15.65
Ca, %	0.71	0.67
available P, %	0.42	0.35
lysine (Lys), %	0.96	1.11
methionine (Met), %	0.27	0.26
Met+Cys, %	0.55	0.52

¹ provided per kg of diet, mg: Cu 10, Fe 80, Mn 30, Zn 80, I 0.5, Se 0.3, vit. K₃ 1.86, vit. B₁ 3, riboflavin 3.6, vit. B₆ 1.5, vitamin B₁₂ 20, pantothenic acid 18, niacin 26, choline 56; IU: vit. A 5850, vit. E 20, vit. D₃ 1251; ² analysed values except digestible energy calculated according to swine National Research Council (NRC, 2012) values

Samples preparation

At day 150 of offspring age, *longissimus dorsi* muscle samples were collected within 24 h after slaughtering. Animal diets, feeding trait and slaughter methods are consistent with our previous study (Guo et al., 2020a,b). Samples preparation of porcine *longissimus dorsi* muscles were according to previous reports (Zhang et al., 2019). The muscle samples were frozen at –20 °C until the geometric centre temperature reached approximately –18 °C. After then, all samples of *longissimus dorsi* muscles were stored at –18 °C for 0, 24, 48, 72 and 96 h, respectively. Six chops for every group (at each storage time) were used for analysis.

Meat colour measurement

The frozen meat samples were thawed in refrigerator at 4 °C for 12 h. And then, the colour of thawed samples (*longissimus dorsi* muscles) was analysed by

colorimeter (Konica Minolta CR 410, Sensing Inc., Osaka, Japan). L^* , a^* and b^* values of meat colour represents lightness, redness and yellowness, respectively (Jia et al., 2012).

Thawing loss, cooking loss and shear force measurement

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

$$\text{thawing loss, \%} = (M_b - M_a) / M_b$$

where: M_b and M_a – weight of the meat sample before and after thawing, respectively.

Thawing meat samples were packed with a plastic bag and kept in 85 °C water for 20 min until the geometric centre temperature reached approximately 75 °C. Cooking loss was calculated according to the percentage weight ratio of meat before and after cooking:

$$\text{cooking loss, \%} = (M_b - M_a) / M_b$$

where: M_b and M_a – weight of thawed meat sample before and after cooking, respectively.

Thawed meat samples were used to determine shear force with a CLM-4 digital explicit muscle tenderness meter (School of Engineering, Northeast Agricultural University, Harbin, China). Shear force of thawed meat was calculated according to previous report (Laville et al., 2007).

Low field nuclear magnetic resonance (LF-NMR)

LF-NMR relaxation time was performed according to the methods as described in previous report (Zhang et al., 2018) using a LF-NMR imaging analyser (NMI20-040V-I; Suzhou Newmai Analytical Instrument Co., Suzhou, China). The thawed meat samples (1.5 cm diameter and 3 cm length of cylindrical tube) were placed into 18-mm cylindrical tubes for analysis. The transverse relaxation time (T_2) was determined by a Carr-Purcell-Meiboom-Gill pulse sequencer (Niumag Electric Corp, Shanghai, China) at 32 °C, 200 μs (time between 90° and 180° pulse), and 22.4 MHz resonance frequency. For each sample 8 scans were carried out at a 3 s-interval with a total of 2000 echoes.

Statistical analysis

Data was analysed using a one-way ANOVA procedure of SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Significant differences among all treatment means were estimated at $P < 0.05$ by Duncan's multiple range tests. The results were presented as mean ± SD (standard deviations).

Results

Meat colour

As shown in Table 3, L^* and b^* values of *longissimus dorsi* muscle in offspring pigs from all groups increased with frozen storage time. At 0 h, L^* and b^* values in HD, ND and LD groups were 54.14, 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^* values in ND group were lower than that of LD group, and were higher than that of HD group during frozen storage. a^* value in all groups decreased with frozen storage time. In HD, ND and LD groups it was 8.23, 8.11, 7.26 at 0 h, and at 96 h decreased to 7.35, 7.08, 6.34, respectively ($P < 0.05$). a^* values in LD group were lower than that in ND and HD groups during frozen storage ($P < 0.05$).

Thawing loss, cooking loss and shear force

As shown in Table 4, thawing loss and cooking loss of *longissimus dorsi* muscles in all groups increased with frozen storage time ($P < 0.05$). Whereas, shear force in all groups decreased with frozen storage time ($P < 0.05$). Thawing loss and shear force in ND group was lower than that of LD group, while was higher than that of HD group during frozen time ($P < 0.05$). Cooking loss in HD group was lower than that in ND and LD groups during frozen storage ($P < 0.05$).

LF-NMR relaxation time

As shown in Table 5 and Figure 1, T_{21} and T_{22} relaxation times of *longissimus dorsi* muscles in all groups increased with frozen storage, whereas, no differences in T_{2a} relaxation times in all groups were observed during frozen storage ($P > 0.05$).

Table 3. Changes in colour of *longissimus dorsi* muscles in offspring pigs during frozen storage

Indices	Treatment	Frozen storage time, h				
		0	24	48	72	96
L^*	HD	54.14 ± 1.17 ^{bC}	56.75 ± 1.80 ^{aC}	56.98 ± 1.37 ^{aC}	57.22 ± 1.09 ^{aC}	57.58 ± 1.16 ^{aC}
	ND	56.97 ± 0.96 ^{bB}	57.18 ± 0.82 ^{abB}	59.06 ± 0.64 ^{abB}	60.14 ± 0.53 ^{abB}	60.64 ± 0.87 ^{abB}
	LD	59.65 ± 0.50 ^{bA}	59.94 ± 0.60 ^{abA}	61.22 ± 0.38 ^{abA}	61.55 ± 0.23 ^{abA}	61.63 ± 0.29 ^{abA}
a^*	HD	8.23 ± 0.13 ^{aA}	8.28 ± 0.12 ^{aA}	7.66 ± 0.15 ^{abA}	7.48 ± 0.13 ^{abA}	7.35 ± 0.12 ^{abA}
	ND	8.11 ± 0.13 ^{aA}	8.16 ± 0.13 ^{aA}	7.31 ± 0.15 ^{baA}	7.21 ± 0.14 ^{baA}	7.08 ± 0.13 ^{baA}
	LD	7.26 ± 0.17 ^{abB}	7.04 ± 0.16 ^{abB}	6.65 ± 0.13 ^{abB}	6.52 ± 0.15 ^{abB}	6.34 ± 0.12 ^{bbB}
b^*	HD	5.85 ± 0.16 ^{cC}	7.32 ± 0.12 ^{bC}	7.49 ± 0.13 ^{bC}	7.74 ± 0.14 ^{abC}	7.95 ± 0.12 ^{abC}
	ND	6.23 ± 0.14 ^{cbB}	7.66 ± 0.17 ^{bbB}	7.95 ± 0.13 ^{abB}	8.35 ± 0.12 ^{abB}	8.67 ± 0.13 ^{abB}
	LD	7.95 ± 0.12 ^{caA}	8.45 ± 0.15 ^{baA}	8.98 ± 0.15 ^{abA}	9.22 ± 0.14 ^{abA}	9.40 ± 0.16 ^{abA}

HD – group fed high level of vitamin D₃ (3200 IU/kg basal diet); ND – group fed normal level of vitamin D₃ (800 IU/kg basal diet); LD – group fed low level of vitamin D₃ (200 IU/kg basal diet); L^* – lightness, a^* – redness, b^* – yellowness; ^{ABC} – means within a column with different capital superscripts are significantly different at $P < 0.05$; ^{abc} – means within a row with different small superscripts are significantly different at $P < 0.05$

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

Indices	Treatment	Frozen storage time, h				
		0	24	48	72	96
Thawing loss, %	HD	1.64 ± 0.11 ^{cC}	1.78 ± 0.22 ^{dC}	1.93 ± 0.14 ^{cC}	2.13 ± 0.16 ^{bC}	2.28 ± 0.21 ^{abC}
	ND	2.11 ± 0.12 ^{abB}	2.31 ± 0.14 ^{dB}	2.44 ± 0.17 ^{cbB}	2.65 ± 0.22 ^{bbB}	2.94 ± 0.23 ^{abB}
	LD	2.35 ± 0.18 ^{aA}	2.57 ± 0.15 ^{dA}	2.81 ± 0.21 ^{caA}	3.12 ± 0.24 ^{baA}	3.38 ± 0.26 ^{abA}
Cooking loss, %	HD	28.84 ± 1.13 ^{bB}	29.29 ± 1.08 ^{abB}	30.56 ± 1.27 ^{abB}	31.22 ± 1.60 ^{abB}	32.04 ± 1.37 ^{abB}
	ND	31.88 ± 0.84 ^{baA}	32.01 ± 0.95 ^{abA}	32.88 ± 0.85 ^{abA}	33.48 ± 0.76 ^{abA}	34.18 ± 0.86 ^{abA}
	LD	32.66 ± 0.98 ^{baA}	32.94 ± 1.05 ^{abA}	33.24 ± 1.08 ^{abA}	33.89 ± 0.95 ^{abA}	34.76 ± 0.71 ^{abA}
Shear force, kg	HD	2.86 ± 0.08 ^{cC}	2.77 ± 0.14 ^{aC}	2.53 ± 0.13 ^{abC}	2.46 ± 0.12 ^{bC}	2.42 ± 0.12 ^{bcC}
	ND	3.31 ± 0.34 ^{abB}	3.14 ± 0.22 ^{abB}	3.02 ± 0.17 ^{abB}	2.99 ± 0.18 ^{bbB}	2.98 ± 0.17 ^{bbB}
	LD	3.58 ± 0.22 ^{aA}	3.46 ± 0.17 ^{aA}	3.38 ± 0.15 ^{abA}	3.42 ± 0.14 ^{abA}	3.26 ± 0.13 ^{baA}

HD – group fed high level of vitamin D₃ (3200 IU/kg basal diet); ND – group fed normal level of vitamin D₃ (800 IU/kg basal diet); LD – group fed low level of vitamin D₃ (200 IU/kg basal diet); ^{ABC} – means within a column with different capital superscripts are significantly different at $P < 0.05$; ^{abc} – means within a row with different small superscripts are significantly different at $P < 0.05$

Table 5. Changes in LF-NMR relaxation times of *longissimus dorsi* muscles in offspring pigs during frozen storage

Indices	Treatment	Frozen storage time, h				
		0	24	48	72	96
T_{2a}	HD	0.50 ± 0.02 ^{abA}	0.55 ± 0.02 ^{abA}	0.53 ± 0.04 ^{abA}	0.54 ± 0.03 ^{abA}	0.54 ± 0.02 ^{abA}
	ND	0.50 ± 0.05 ^{abA}	0.53 ± 0.03 ^{abA}	0.54 ± 0.02 ^{abA}	0.55 ± 0.02 ^{abA}	0.55 ± 0.03 ^{abA}
	LD	0.50 ± 0.01 ^{abA}	0.51 ± 0.02 ^{abA}	0.56 ± 0.02 ^{abA}	0.56 ± 0.02 ^{abA}	0.56 ± 0.03 ^{abA}
T_{21}	HD	13.56 ± 0.69 ^{bbB}	15.22 ± 1.42 ^{abbB}	15.58 ± 1.30 ^{abbB}	15.96 ± 0.75 ^{abB}	16.14 ± 0.85 ^{abB}
	ND	14.17 ± 1.17 ^{baA}	15.99 ± 0.58 ^{abA}	16.42 ± 0.55 ^{abA}	16.81 ± 0.96 ^{abA}	16.94 ± 1.11 ^{abA}
	LD	14.18 ± 1.31 ^{baA}	16.14 ± 0.66 ^{abA}	16.38 ± 0.60 ^{abA}	16.89 ± 0.52 ^{abA}	17.21 ± 0.56 ^{abA}
T_{22}	HD	167.17 ± 0.96 ^{bcC}	230.32 ± 9.17 ^{abbB}	234.45 ± 9.60 ^{abbB}	241.18 ± 7.50 ^{abC}	248.47 ± 8.72 ^{abC}
	ND	183.48 ± 8.06 ^{cbB}	231.16 ± 10.14 ^{bbB}	235.09 ± 10.33 ^{bbB}	269.95 ± 11.20 ^{abbB}	291.18 ± 9.73 ^{abB}
	LD	192.20 ± 8.33 ^{caA}	262.62 ± 8.73 ^{baA}	272.23 ± 10.97 ^{baA}	301.35 ± 13.42 ^{abbA}	324.42 ± 7.49 ^{abA}

HD – group fed high level of vitamin D₃ (3200 IU/kg basal diet); ND – group fed normal level of vitamin D₃ (800 IU/kg basal diet); LD – group fed low level of vitamin D₃ (200 IU/kg basal diet); ^{ABC} – means within a column with different capital superscripts are significantly different at $P < 0.05$, ^{abc} – means within a row with different small superscripts are significantly different at $P < 0.05$

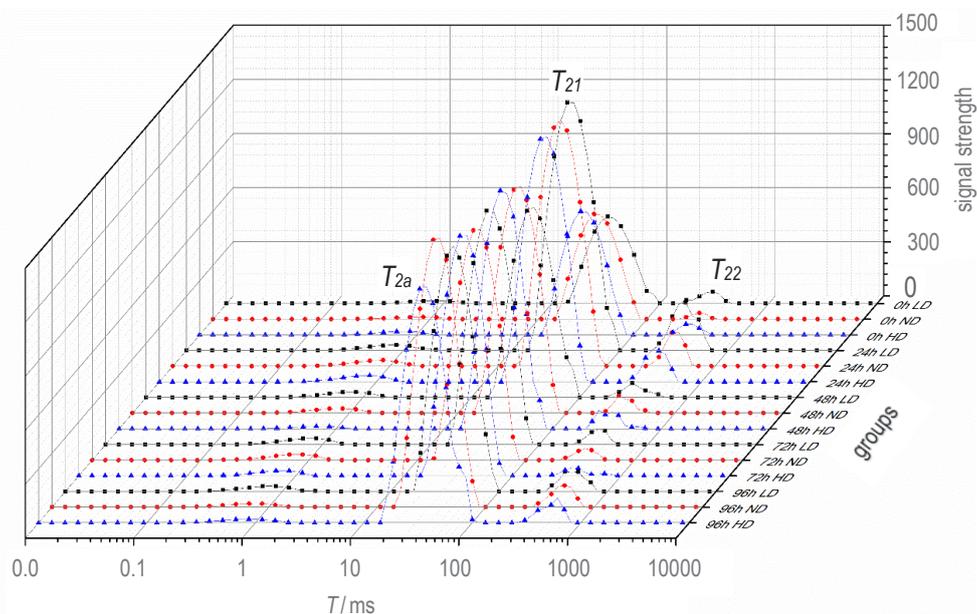


Figure 1. Three-dimensional T_{2a} relaxation time plot of *longissimus* muscles in offspring pigs from LD, ND and HD groups during frozen storage; HD – group fed high level of vitamin D₃ (3200 IU/kg basal diet); ND – group fed normal level of vitamin D₃ (800 IU/kg basal diet); LD – group fed low level of vitamin D₃ (200 IU/kg basal diet)

T_{21} relaxation times in HD group were lower than that in ND and LD groups ($P < 0.05$). T_{22} relaxation times in ND group were lower than that in LD group, and were higher than that in HD group during frozen storage ($P < 0.05$). There was no difference in T_{2a} relaxation times among HD, ND and LD groups during frozen storage ($P > 0.05$).

Discussion

Meat colour. Meat colour usually affects consumer's purchasing decisions, and is used for assessing freshness and attributes of meat quality

(Uhlířová et al., 2018). In this study, L^* and b^* values of *longissimus dorsi* muscle in offspring pigs from all groups increased with the prolongation of frozen storage time. Whereas, a^* value decreased with extended frozen storage time. These results suggested that meat colour was affected by frozen storage time. Similar results were observed by Zhang et al. (2019), who found that meat colour of porcine *longissimus* muscles 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. It was also found that there is a negative

correlation between the a^* value of frozen muscle and frozen storage time. It may be due to the decrease metmyoglobin-reducing enzymes activity with prolonged frozen storage (Farouk and Swan, 1998). Whereas, L^* value increased with frozen storage time. It may be due to the increase of muscle fibre contraction during freezing, which reduced the light scattering on the meat surface (Hector et al., 1992). In this study, b^* value also increased with frozen storage time. Similar results were reported by Zhang et al., (2019), which may be related to increase in protein oxidation and thiobarbituric acid-reactive substance during frozen storage.

In this study we have found that in comparison with HD group, in LD group higher L^* and b^* values, while lower a^* values of *longissimus dorsi* muscles at whole frozen storage periods were observed. These results indicated that maternal vitamin D₃ status may influenced meat colour of frozen muscles in offspring, and maternal vitamin D₃ deficiency decreased sensory quality of meat. It was previously found that pork colour discoloration is associated with pigment and lipid oxidation (Mitsumoto et al., 1993). In our study it may be due to maternal vitamin D₃ status which changed the activity of the oxidative processes and pigment oxidation in *longissimus dorsi* muscles, which influenced a^* , L^* and b^* values of pork of offspring pigs. Similar results were observed by Duffy et al. (2018), who found that dietary vitamin D₂ decreased lipid peroxidation of *longissimus thoracis* steak of pigs, improved redness value, and colour stability. Whereas, in other study it was found that supplementation of vitamin D₃ did not influence a^* and b^* values, while decreased L^* value of pork in finishing pigs (Wilborn et al., 2004). Inconsistent research results in meat colour might be due to species, ages, dosage and duration of vitamin D₃ feeding.

Thawing loss, cooking loss and shear force.

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 (Leygonie et al., 2012). It was demonstrated that thawing loss usually affects the colour and sensory quality of meat (Xia et al., 2009), and is associated with the destruction of muscle fibre structure and the denaturation of protein (Leygonie et al., 2012). In our present study, the thawing loss of all groups increased with frozen storage, which may be due to the fracture of muscle fibres caused by the formation of ice crystals at frozen storage (Rahelic et al., 1985). These results suggested

that thawing loss of *longissimus dorsi* muscle in offspring pigs were affected by frozen storage time. Similar results were observed by Zhang et al. (2019), who found that thawing loss of porcine *longissimus* muscles (air freezing, immersion freezing and ultrasound-assisted immersion freezing) increased with frozen storage time. We have found for the first time that thawing loss in offspring pigs from LD group was significantly higher than that from HD group, which indicated that maternal vitamin D₃ supplementation may reduce the formation of ice crystals, and decrease damage of the muscle structure during frozen storage, and improve water holding capacity and meat quality attributes in offspring pigs. In addition, maternal vitamin D₃ deficiency increased destruction of muscle structure and decreased the water binding capacity of muscle in offspring pigs. These results indicated that after thawing, in comparison with LD group, the muscle samples from 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 (Vieira et al., 2009). There is a negative correlation between cooking loss and eating quality of meat (Aaslyng et al., 2003). In this study, cooking loss of *longissimus dorsi* muscle from all groups increased with frozen storage time. It can be suggested that the quality of meat can still be lost during the process of frozen storage. Similar results were observed by Zhang et al. (2019), who found that cooking loss of porcine *longissimus* muscles significantly increased with the increase in frozen storage time. In HD group the lowest cooking loss during frozen storage was noted, which indicated that maternal vitamin D₃ supplementation could prolong and protect the porcine meat quality through inhibiting the decrease in cooking loss of *longissimus dorsi* muscle in offspring pigs. It can be supposed that maternal vitamin D₃ supplementation maintained the integrity of muscle tissue and decreased cooking loss in offspring pigs. However, the underlying mechanism still needs to be investigated in the future.

Shear force usually reflects the tenderness of meat, and the increase in tenderness is associated to the length of frozen storage (Leygonie et al., 2012). In this present study, shear force of *longissimus dorsi* muscle from all groups decreased with frozen storage time. Similar results were observed by

Kim et al. (2013), who found that shear force of freeze-thawed pork decreased with storage time. However, it was also observed that shear force of beef decreased with frozen storage (Farouk et al., 2004; Lagerstedt et al., 2008). Whereas, it is found that freezing did not influenced shear force of beef (Vieira et al., 2009). Inconsistent research results in shear force value might be due to species, frozen storage time and temperature. It was previously demonstrated that shear force is negatively correlated to IMF content of muscle (Magnabosco et al., 2016). In this study, shear force in offspring pigs from LD group were significantly higher than that from HD group, which suggested that maternal vitamin D₃ deficiency may decreased the tenderness of *longissimus dorsi* muscle in offspring pigs during frozen storage through inhibiting the formation of IMF (Guo et al., 2020b). These results indicated that maternal vitamin D₃ status could affect the meat quality attributes in offspring pigs during frozen storage.

LF-NMR. 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 (Zhang et al., 2017b). 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 Zhang et al. (2017a), who observed that T_{21} relaxation times of porcine *longissimus* muscle increased with the increased freeze-thaw cycles, 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 (Sanchez-Alonso et al., 2012). 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 (Leygonie et al., 2012). In previous studies it was found that relaxation time is correlated to meat quality attributes, and higher T_2 relaxation time usually reflects higher thawing loss (Renou et al., 1985; Zhang et al., 2019). We have 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 from HD group were significantly lower than that from LD group, which indicated that maternal vitamin D₃ supplementation could increase water holding capacity and meat quality in offspring pigs. Whereas, maternal vitamin D₃ deficiency increased thawing loss and decreased water holding capacity of offspring pigs during frozen storage.

Conclusions

Maternal vitamin D₃ status significantly affected meat quality attributes and water distribution of *longissimus dorsi* muscles in offspring pigs during frozen storage. Values of L^* , b^* , thawing loss, cooking loss, T_{21} and T_{22} relaxation times of *longissimus dorsi* muscles in all offspring pigs from mothers fed 200, 800, and 3200 IU of vitamin D₃/kg basal diet during pregnancy increased with frozen storage, whereas, a^* value and shear force decreased with frozen storage. In addition, maternal vitamin D₃ supplementation could improve meat quality and water holding capacity of offspring pigs during frozen storage.

Acknowledgements

This study was supported by grants from the Henan joint funds of National Natural Science Foundation of China (U1604102), the National Natural Science Foundation of China (31572417), and Provincial key Technology Research and Development Program of Henan (192102110069).

References

- Aaslyng M.D., Bejerholm C., Ertbjerg P., Bertram H.C., Andersen H.J., 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Qual. Prefer.* 14, 277–288, [https://doi.org/10.1016/S0950-3293\(02\)00086-1](https://doi.org/10.1016/S0950-3293(02)00086-1)
- Abrams G.D., Feldman D., Safran M.R., 2018. Effects of vitamin D on skeletal muscle and athletic performance. *J. Am. Acad. Orthop. Surg.* 26, 278–285, <https://doi.org/10.5435/JAAOS-D-16-00464>
- Bauer A., Scheier R., Eberle T., Schmidt H., 2016. Assessment of tenderness of aged bovine gluteus medius muscles using Raman spectroscopy. *Meat Sci.* 115, 27–33, <https://doi.org/10.1016/j.meatsci.2015.12.020>
- D'Souza D.N., Mullan B.P., 2002. The effect of genotype, sex and management strategy on the eating quality of pork. *Meat Sci.* 60, 95–101, [https://doi.org/10.1016/S0309-1740\(01\)00112-7](https://doi.org/10.1016/S0309-1740(01)00112-7)
- Duffy S.K., Kelly A.K., Rajauria G., et al., 2018. The use of synthetic and natural vitamin D sources in pig diets to improve meat quality and vitamin D content. *Meat Sci.* 143, 60–68, <https://doi.org/10.1016/j.meatsci.2018.04.014>

- Farouk M.M., Swan J.E., 1998. Effect of rigor temperature and frozen storage on functional properties of hot-boned manufacturing beef. *Meat Sci.* 49, 233–247, [https://doi.org/10.1016/S0309-1740\(97\)00134-4](https://doi.org/10.1016/S0309-1740(97)00134-4)
- Farouk M.M., Wieliczko K.J., Merts I., 2004. Ultra-fast freezing and low storage temperatures are not necessary to maintain the functional properties of manufacturing beef. *Meat Sci.* 66, 171–179, [https://doi.org/10.1016/S0309-1740\(03\)00081-0](https://doi.org/10.1016/S0309-1740(03)00081-0)
- Fernandez X., Monin G., Talmant A., Mourot J., Lebret B., 1999. Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of *m. longissimus lumborum*. *Meat Sci.* 53, 67–72, [https://doi.org/10.1016/S0309-1740\(99\)00038-8](https://doi.org/10.1016/S0309-1740(99)00038-8)
- Guo L., Miao Z., Ma H., Melnychuk S., 2020a. Effects of maternal vitamin D₃ concentration during pregnancy on adipogenic genes expression and serum biochemical index in offspring piglets. *J. Anim. Feed Sci.* 29, 125–131, <https://doi.org/10.22358/jafs/124041/2020>
- Guo L., Miao Z., Ma H., Melnychuk S., 2020b. Effects of maternal vitamin D₃ during pregnancy on *FASN* and *LIPE* mRNA expression in offspring pigs. *J. Agric. Sci.* 158, 128–135, <https://doi.org/10.1017/S0021859620000210>
- Hector D.A., Brew-Graves C., Hassen N., Ledward D.A., 1992. Relationship between myosin denaturation and the colour of low-voltage-electrically-stimulated beef. *Meat Sci.* 31, 299–307, [https://doi.org/10.1016/0309-1740\(92\)90060-H](https://doi.org/10.1016/0309-1740(92)90060-H)
- Houbak M.B., Ertbjerg P., Therkildsen M., 2008. *In vitro* study to evaluate the degradation of bovine muscle proteins *post-mortem* by proteasome and mu-calpain. *Meat Sci.* 79, 77–85, <https://doi.org/10.1016/j.meatsci.2007.08.003>
- Hunt M.R., Garmyn A.J., O'Quinn T.G., Corbin C.H., Legako J.F., Rathmann R.J., Brooks J.C., Miller M.F., 2014. Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. *Meat Sci.* 98, 1–8, <https://doi.org/10.1016/j.meatsci.2014.04.004>
- Jia N., Kong B.H., Liu Q., Diao X.P., Xia X.F., 2012. Antioxidant activity of black currant (*Ribes nigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Sci.* 91, 533–539, <https://doi.org/10.1016/j.meatsci.2012.03.010>
- Kim G.D., Jung E.Y., Lim H.J., Yang H.S., Joo S.T., Jeong J.Y., 2013. Influence of meat exudates on the quality characteristics of fresh and freeze-thawed pork. *Meat Sci.* 95, 323–329, <https://doi.org/10.1016/j.meatsci.2013.05.007>
- Lagerstedt A., Enfalt L., Johansson L., Lundstrom K., 2008. Effect of freezing on sensory quality, shear force and water loss in beef M. longissimus dorsi. *Meat Sci.* 80, 457–461, <https://doi.org/10.1016/j.meatsci.2008.01.009>
- Laville E., Sayd T., Terlouw C., Chambon C., Damon M., Larzul C., Leroy P., Glenisson J., Cherel P., 2007. Comparison of sarcoplasmic proteomes between two groups of pig muscles selected for shear force of cooked meat. *J. Agric. Food. Chem.* 55, 5834–5841, <https://doi.org/10.1021/jf070462x>
- Leygonie C., Britz T.J., Hoffman L.C., 2012. Impact of freezing and thawing on the quality of meat: review. *Meat Sci.* 91, 93–98, <https://doi.org/10.1016/j.meatsci.2012.01.013>
- Magnabosco C.U., Lopes F.B., Fragozo R.C., Eifert E.C., Valente B.D., Rosa G.J., Sainz R.D., 2016. Accuracy of genomic breeding values for meat tenderness in Polled Nellore cattle. *J. Anim. Sci.* 94, 2752–2760, <https://doi.org/10.2527/jas.2016-0279>
- Miao Z.G., Zhang L.P., Fu X., Yang Q.Y., Zhu M.J., Dodson M.V., Du M., 2016. Invited review: mesenchymal progenitor cells in intramuscular connective tissue development. *Animal* 10, 75–81, <https://doi.org/10.1017/S1751731115001834>
- Mitsumoto M., Arnold R.N., Schaefer D.M., Cassens R.G., 1993. Dietary versus postmortem supplementation of vitamin E on pigment and lipid stability in ground beef. *J. Anim. Sci.* 71, 1812–1816, <https://doi.org/10.2527/1993.7171812x>
- Mottram D.S., 1998. Flavour formation in meat and meat products: a review. *Food Chem.* 62, 415–424, [https://doi.org/10.1016/S0308-8146\(98\)00076-4](https://doi.org/10.1016/S0308-8146(98)00076-4)
- NRC (National Research Council), 2012. Nutrient Requirements of Swine. 11th Revised Edition. The National Academies Press, Washington, DC (USA), <https://doi.org/10.17226/13298>
- Rahelic S., Puac S., Gawwad A.H., 1985. Structure of beef *Longissimus dorsi* muscle frozen at various temperatures: Part 1-histological changes in muscle frozen at -10, -22, -33, -78, -115 and -196°C. *Meat Sci.* 14, 63–72, [https://doi.org/10.1016/0309-1740\(85\)90082-8](https://doi.org/10.1016/0309-1740(85)90082-8)
- Renou J.P., Monin G., Sellier P., 1985. Nuclear magnetic resonance measurements on pork of various qualities. *Meat Sci.* 15, 225–233, [https://doi.org/10.1016/0309-1740\(85\)90078-6](https://doi.org/10.1016/0309-1740(85)90078-6)
- Sanchez-Alonso I., Martinez I., Sanchez-Valencia J., Careche M., 2012. Estimation of freezing storage time and quality changes in hake (*Merluccius merluccius* L.) by low field NMR. *Food Chem.* 135, 1626–1634, <https://doi.org/10.1016/j.foodchem.2012.06.038>
- Trout G.R., 1988. Techniques for measuring water-binding capacity in muscle foods-A review of methodology. *Meat Sci.* 23, 235–252, [https://doi.org/10.1016/0309-1740\(88\)90009-5](https://doi.org/10.1016/0309-1740(88)90009-5)
- Uhlírova L., Tumova E., Chodova D., Vlckova J., Ketta M., Volek Z., Skrivanova V., 2018. The effect of age, genotype and sex on carcass traits, meat quality and sensory attributes of geese. *Asian-Australas. J. Anim. Sci.* 31, 421–428, <https://doi.org/10.5713/ajas.17.0197>
- Ventanas S., Ruiz J., Garcia C., Ventanas J., 2007. Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Sci.* 77, 324–330, <https://doi.org/10.1016/j.meatsci.2007.04.001>
- Vieira C., Diaz M.T., Martinez B., Garcia-Cachan M.D., 2009. Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Sci.* 83, 398–404, <https://doi.org/10.1016/j.meatsci.2009.06.013>
- Wilborn B.S., Kerth C.R., Owsley W.F., Jones W.R., Frobish L.T., 2004. Improving pork quality by feeding supranutritional concentrations of vitamin D₃. *J. Anim. Sci.* 82, 218–224, <https://doi.org/10.2527/2004.821218x>
- Xia X., Kong B., Liu Q., Liu J., 2009. Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. *Meat Sci.* 83, 239–245, <https://doi.org/10.1016/j.meatsci.2009.05.003>
- Zhang M., Haili N., Chen Q., Xia X., Kong B., 2018. Influence of ultrasound-assisted immersion freezing on the freezing rate and quality of porcine longissimus muscles. *Meat Sci.* 136, 1–8, <https://doi.org/10.1016/j.meatsci.2017.10.005>
- Zhang M., Xia X., Liu Q., Chen Q., Kong B., 2019. Changes in microstructure, quality and water distribution of porcine longissimus muscles subjected to ultrasound-assisted immersion freezing during frozen storage. *Meat Sci.* 151, 24–32, <https://doi.org/10.1016/j.meatsci.2019.01.002>
- Zhang M.C., Li F.F., Diao X.P., Kong B.H., Xia X.F., 2017a. Moisture migration, microstructure damage and protein structure changes in porcine longissimus muscle as influenced by multiple freeze-thaw cycles. *Meat Sci.* 133, 10–18, <https://doi.org/10.1016/j.meatsci.2017.05.019>
- Zhang Z., Regenstein J.M., Zhou P., Yang Y., 2017b. Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel. *Ultrason. Sonochem.* 34, 960–967, <https://doi.org/10.1016/j.ultsonch.2016.08.008>
- Zhou H., Chen Y.L., Lv G., Zhuo Y., Lin Y., Feng B., Fang Z.F., Che L.Q., Li J., 2016. Improving maternal vitamin D status promotes prenatal and postnatal skeletal muscle development of pig offspring. *Nutrition* 32, 1144–1152, <https://doi.org/10.1016/j.nut.2016.03.004>