



## RESEARCH ARTICLE

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# Effects of dietary vitamin levels on physiological responses, blood profiles, and reproductive performance in gestating sows

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## Abstract

This study was performed to evaluate the effects of dietary vitamin levels on physiological responses, blood profiles, and reproductive performance in gestating sows. A total of 52 F1 multiparous sows (Yorkshire × Landrace) with an average body weight of  $223.5 \pm 31.7$  kg, an average parity of  $6.4 \pm 2.7$ , and an average backfat thickness of  $18.5 \pm 4.9$  mm were divided into four treatment groups considering body weight, backfat thickness, and parity in a completely randomized design with 13 replicates. The treatments were 100% (V1), 300% (V3), 600% (V6) and 900% (V9) of the National Research Council (NRC) *Nutrient Requirements of Swine*. The gestation diet was formulated based on corn-soybean meal (SBM) and contained 3,265 kcal of metabolizable energy (ME)/kg and 12.00% crude protein. During the lactation period, all sows were fed the same commercial lactation diet. There was no significant difference in body weight of gestating sows. However backfat thickness tended to increase when higher levels of vitamins were provided to gestating sows ( $p < 0.10$ ). When high levels of dietary vitamins were provided, the body weight change of lactating sows increased ( $p < 0.01$ ). When sows were fed higher levels of vitamins, the feed intake of lactating sows tended to decrease ( $p = 0.06$ ). There were no treatment differences in the number of total born, born alive, stillbirth piglets, or the body weight of piglets according to different dietary vitamin level. As dietary vitamin level increased, the serum concentration of 25(OH)D<sub>3</sub> in sows at 90 days of gestation linearly increased ( $p < 0.01$ ). Furthermore, the serum vitamin E level of gestating sows was linearly increased with increasing dietary vitamin level ( $p < 0.05$ ). The current NRC vitamin requirements are sufficient for gestating sows and higher levels of vitamins in the gestation diet did not show any beneficial effects for gestating and lactating sows.

**Keywords:** Gestating sow, Litter performance, Reproductive performance, Serum vitamin concentration, Vitamin levels

## Background

Vitamins are organic compounds required in only small quantities for normal growth, maintenance, and reproduction of animal life. Vitamin D<sub>3</sub> is essential for the growth of, and maintenance of calcium and phosphorus homeostasis in, mammals [1]. In pigs, during gestation and lactation periods, vitamin D is required for

calcium metabolism, to promote fetal growth and superior milk composition [2]. Vitamin E supplementation has been shown to increase the immunogenic capacity of reproductive sows [3], which is important for embryonic development and survival [4–6]. Vitamin E deficiency has been shown to affect reproduction in several animals, resulting in fetal death and resorption [7]. Therefore, a number of studies have been performed to estimate the vitamin

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requirements of pigs. Lindermann et al. [8] reported increasing trends of weight gain and feed intake in weanling pigs fed vitamins at levels five times higher than those suggested by the National Research Council (NRC) [9], but feed efficiency tended to be reduced with higher vitamin levels. Boyd et al. [10] reported that vitamin supplementation improved the litter size of sows. However, there have been few studies of the vitamin D requirements of sows during gestation or lactation [9].

Pig diets can be supplemented with a vitamin premix product in Korea to meet the requirements of the animals [11,12]. Flohr et al. [13] studied vitamins and minerals in the diet currently used in US swine farms, in a total of about 2,268,900 sows. They reported that pig producers added fat-soluble vitamins to the gestation diet at levels that were 2.6-, 2.2-, 1.6-, and 7.3-fold higher than the NRC requirements for vitamins A, D, E and K, respectively. Most feed companies in Korea supplement pig diets with vitamin premix at levels that are 3–10-fold higher than the NRC requirements [11]. The excessive vitamin supplementation of the diet of sows is responsible for the rising feed costs in Korea.

This study was performed to evaluate the effects of dietary vitamin levels on physiological responses, blood profiles, and reproductive performance in gestating sows.

## Materials and Methods

### Animals

All experimental procedures involving animals were performed in accordance with the Animal Experimental Guidelines of the Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC; SNU-160819-11). A total of 52 F1 multiparous sows (Yorkshire × Landrace) with an average body weight of  $223.5 \pm 31.7$  kg, an average parity of  $6.4 \pm 2.7$  and an av-

erage backfat thickness of  $18.5 \pm 4.9$  mm were moved to individual gestation stalls for artificial insemination (AI). The sows were divided into one of four treatment groups according to body weight, backfat thickness, and parity in a completely randomized design with 13 replicates. They had twice-daily contact with a boar. AI was performed twice a day at 12-hour intervals with fresh diluted semen (Darby A.I. Center, Anseong, Korea) when signs of first estrus were detected. Pregnancy was checked at day 35 of gestation using an ultrasound scanner (Dongjin BLS, Gwangju, Korea).

### Experimental design and diet

All experimental diets for gestating sows were based on corn-soybean meal (SBM) supplemented with vitamin premix at various levels; the treatments were 100% (V1), 300% (V3), 600% (V6), and 900% (V9) of the NRC (2012) requirements.

Experimental vitamin premix was formulated to meet the NRC requirements (2012) when supplemented in the feed at 0.033%. The vitamin premix formulas in the gestation diet are shown in Table 1. All experimental diets were supplemented with 0.1% choline chloride, and were formulated to contain 13.67 MJ of metabolizable energy/kg, 12.00% crude protein, 0.23% methionine, 0.74% lysine, 0.75% calcium, and 0.60% total phosphorus. All other nutrients were formulated to meet or exceed the NRC requirements (2012). Table 2 shows the formulas and chemical compositions of the experimental diets. All sows were fed the same commercial lactation diet during the lactation period.

### Animal management

All experimental sows were fed the appropriate experimental diet once a day at around 08:00, and provided with 2.4 kg/day during gestation period. All sows were accommodated in individual gestation stalls (2.20 × 0.65 m) and the temperature was maintained at

**Table 1.** Formulas and concentrations of vitamin premix in gestation diets

	Vitamin requirements of the NRC (2012)	Concentration of vitamin in vitamin premix
Vitamin A (IU/kg)	4,000	12,000
Vitamin D <sub>3</sub> (IU/kg)	800	2,400
Vitamin E (50%) (IU/kg)	44	132
Vitamin K (menadione) (mg/kg)	0.50	1.50
Biotin (2%) (mg/kg)	0.20	0.60
Folacin (vitamin B <sub>9</sub> ) (mg/kg)	1.30	3.90
Niacin (mg/kg)	10	30
Calcium d-pantothenate (mg/kg)	12	36
Riboflavin (vitamin B <sub>2</sub> ) (80%) (mg/kg)	3.75	11.25
Thiamin (vitamin B <sub>1</sub> ) (98%) (mg/kg)	1.00	3.00
Vitamin B <sub>6</sub> (100%) (mg/kg)	1.00	3.00
Vitamin B <sub>12</sub> (1%) (µg/kg)	15	45

NRC, National Research Council.

**Table 2. Formulas and chemical compositions of gestation diets**

Item	Gestation diet <sup>1)</sup>			
	V1	V3	V6	V9
<b>Ingredients (%)</b>				
Corn	76.897	76.77	76.58	76.38
Soybean meal-46	11.97	11.99	12.02	12.05
Wheat bran	6.00	6.00	6.00	6.00
Tallow	1.75	1.79	1.85	1.92
L-lysine HCl (78%)	0.26	0.26	0.26	0.26
DL-methionine (99%)	0.04	0.04	0.04	0.04
DCP	1.35	1.35	1.35	1.35
Limestone	1.20	1.20	1.20	1.20
Vit. premix <sup>2)</sup>	0.033	0.10	0.20	0.30
Min. premix <sup>3)</sup>	0.10	0.10	0.10	0.10
Choline chloride-50	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
<b>Chemical composition<sup>4)</sup></b>				
ME (MJ/kg)	13.67	13.67	13.67	13.67
Crude protein (%)	12.00	12.00	12.00	12.00
Total lysine (%)	0.74	0.74	0.74	0.74
Total methionine (%)	0.23	0.23	0.23	0.23
Calcium (%)	0.75	0.75	0.75	0.75
Total phosphorus (%)	0.60	0.60	0.60	0.60

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM)-based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement.

<sup>2)</sup>Provided per kg of diet: vitamin A, 12,000 IU; vitamin D<sub>3</sub>, 2,400 IU; vitamin E, 132 IU; vitamin K, 1.5 mg; biotin, 0.60 mg; folacin, 3.9 mg; niacin, 30 mg; calcium pantothenic acid, 36 mg; thiamin, 3 mg; vitamin B<sub>6</sub>, 3 mg; vitamin B<sub>12</sub>, 45 g.

<sup>3)</sup>Provided per kg of diet: Se, 0.3 mg; I, 0.3 mg; Mn, 49 mg; Cu, 288 mg; Fe, 281 mg; Zn, 84.7 mg; Co, 0.3 mg.

<sup>4)</sup>Calculated value.

DCP, digestible crude protein; ME, metabolizable energy.

an average of 20°C by an automated ventilation system. Pregnant sows were washed and moved into farrowing crates (2.40 × 1.80 m) on day 110 of gestation and the gestation diet was decreased gradually by 0.2 kg per day over the 5 days before farrowing. Delivery inducer was not used during farrowing and assistance was provided in all cases of dystocia. After farrowing, the lactation diet was increased gradually from 1.0 kg/day until 5 days postpartum, and then provided *ad libitum* during the lactation period. Each farrowing crate was equipped with a feeder and a nipple waterer for sows, and a heat lamp for newborn piglets. The temperature of the lactation barn was kept at 28°C ± 2°C and the piglet area was maintained at 32°C ± 2°C under a heating lamp. The air condition in the lactation barn was regulated automatically by a ventilation system and air-conditioner. Piglets were cross-fostered within treatment

groups until 24 hours postpartum, to balance the suckling intensity of sows with litter size, and thus to minimize any potential effect of initial litter size on litter growth. Cutting of the umbilical cord and tail and castration were performed 3 days after birth, and piglets were injected with 150 ppm Fe-dextran (Gleptosil®; Alstoe, York, UK). None of the piglets were fed creep feed during the whole lactation period. Weaning was performed at approximately 21 days after birth.

**Body weight, backfat thickness, lactation feed intake**

The live body weight and backfat thickness of sows were recorded at mating, at 70, 90, and 110 days of gestation, at 24 hours postpartum, and at 21 days of lactation, respectively. The body weight of sows was measured using an electronic scale (CAS Co. Ltd., Yangju-si, Gyeonggi-do, Korea) and backfat thickness was measured using an ultrasound device (Lean Meter®; Renco Corp., Minneapolis, MN, USA) at the P2 position (mean value from both sides of the last rib and 65 mm from the backbone). Daily feed wastage was recorded during the lactation period, and lactation feed intake was estimated for 21 days to determine the physiological effects on sows.

**Reproductive performance**

The reproductive traits were recorded within 24 hours postpartum, including total number of piglets, number of piglets born alive, stillbirths, and piglet losses. Individual piglet weights (born and stillbirth) were measured at birth and 21 days of lactation using an electronic scale (CAS Co. Ltd.). Ear notching was performed when measuring the body weight of the piglets. The average daily weight gain of the piglets was calculated to determine their growth and lactation performance after farrowing. The weaning to estrus interval (WEI) of sows, as an important parameter for evaluating reproductive performance, was measured after weaning.

**Blood profiles**

Blood samples (n = 4 for each treatment) were collected from the jugular vein of sows using 10-mL disposable syringes at mating, days 70, 90, and 110 of gestation, 24 hours postpartum, and 21 days of lactation. In addition, blood samples were collected from the anterior vena cava of piglets using 3-mL disposable syringes at 24 hours postpartum, and 5-mL disposable syringes at 21 days of lactation. All blood samples were collected into serum tubes (BD Vacutainer SST™ II Advance; Becton Dickinson, Plymouth, UK) and EDTA tubes (BD Vacutainer K2E; Becton Dickinson). Individual samples were centrifuged at 2,515 × g, 4°C for 15 minutes (5810R; Eppendorf, Hamburg, Germany) and the supernatants were separated into microtubes (Axygen, Union City, CA, USA) and stored at -20°C until analysis.

Serum 25-(OH)-vitamin D was analyzed by chemiluminescence immunoassay (CIA) (Liaison; DiaSorin, Cypress, CA, USA), vitamin E by high-performance liquid chromatography (HPLC) (HPLC-UVD; Waters Corporation, Milford, MA, USA), calcium by colorimetry (Hitachi, Tokyo, Japan), and inorganic phosphorus by UV spectrophotometry (Roche Diagnostics, Penzberg, Germany).

### Statistical analysis

All data were analyzed by analysis of variance (ANOVA) in a completely randomized design using the GLM procedure in SAS software (ver.; SAS Institute, Cary, NC, USA). Orthogonal polynomial contrast was used to determine linear and quadratic effects according to increases in the vitamin levels of the gestation diets of sows and piglets. Individual sows and their litters were used as the experimental unit for analyses of growth performance, reproductive performance and blood profile. Differences among means were taken to be significant at  $p < 0.05$  and highly significant at  $p < 0.01$ , with trends being between  $p \geq 0.05$  and  $p < 0.10$ . When significance was detected, Fisher's least significant difference (LSD) test was applied for post hoc analysis.

## Results and Discussion

### Body weight, backfat thickness of gestating sows

The effects of different levels of vitamin supplementation on the body weight and backfat thickness of sows during the gestation period are shown in Table 3. There were no significant group differences in terms of the body weight of gestating sows at any

time point examined. Sows fed higher levels of vitamins tended to show increased backfat thickness, both at 90 days of gestation and over the whole gestation period (linear,  $p = 0.067$ ,  $p = 0.081$ , respectively). Lauridsen et al. [14] reported no differences in body weight change during the first 28 days of gestation in gilts fed diets varying in vitamin D (vitamin D<sub>3</sub> or 25-(OH)-vitamin D) level (200, 800, 1,400, or 2,000 IU/kg of diet). Similarly, vitamin D<sub>3</sub> supplementation (concentrations between 1,500 and 6,000 IU/kg) of the complete diet had no influence on sow body weight change or average daily feed intake (ADFI) during the lactation period [15]. Mahan [16] reported that dietary vitamin E level had no effect on the body weight of sows given diets supplemented with vitamin E (22, 44, or 66 IU/kg of diet) during the gestation and lactation periods. In the present study, no significant difference was observed in the body weight of gestating sows fed diets containing vitamins D and E at various concentrations (800, 2,400, 4,800, or 7,200 IU/kg of diet and 44, 132, 264, or 396 IU/kg of diet, respectively). Mahan [16] reported no significant differences in backfat thickness at 109 days post coitum or weaning between sows fed different levels of vitamin E. In addition, Lauridsen et al. [14] reported that vitamin D dose had no influence on backfat thickness in sows. Shelton et al. [17] observed no differences in sow body weight or backfat thickness during the entire period from gestation to lactation, when sows were fed diets supplemented with vitamin E at four levels (11, 22, 33, or 44 IU/kg of diet).

### Body weight, backfat thickness, daily feed intake and WEI of lactating sows

The effects of different vitamin levels in the diet during the ges-

**Table 3.** Effects of vitamin supplementation levels in gestation diets on growth performance during gestation

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
Body weight (kg)							
At mating	223.76	223.23	223.73	223.23	4.388	0.978	0.998
70 days	250.54	242.77	241.95	243.87	3.899	0.576	0.549
90 days	259.04	252.05	253.00	254.14	3.878	0.711	0.616
110 days	269.69	267.59	267.21	268.68	4.157	0.932	0.838
BW gain (0–110 days)	45.90	44.36	43.48	45.45	1.455	0.101	0.965
Backfat thickness (mm)							
At mating	18.65	18.35	18.46	18.46	0.674	0.941	0.912
70 days	20.35	22.70	21.04	23.14	0.899	0.432	0.943
90 days	21.15	23.32	23.00	26.80	0.971	0.067	0.673
110 days	22.50	22.95	24.32	26.60	0.960	0.129	0.639
BF gain (0–110 days)	3.85	4.60	5.86	8.14	0.533	0.081	0.165

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM) based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement. Lin., linear; Quad., quadratic; BW, body weight; BF, backfat thickness.

tation period on body weight, backfat thickness, daily feed intake, and WEI during the lactation period are presented in Table 4. There were no significant differences in body weight at 24 hours postpartum or 21 days of lactation among the treatment groups. On the other hand, the body weight change of lactating sows was increased when they were provided with diets with higher vitamin levels during the gestation period (linear,  $p = 0.027$ ). There was no significant difference in backfat thickness at 24 hours postpartum or 21 days of lactation among the treatment groups. The feed intake of lactating sows tended to decrease with increasing vitamin levels in the diet (linear,  $p = 0.06$ ). After weaning, the WEI showed a quadratic response as the vitamin supplementation level was increased (quadratic,  $p = 0.047$ ), although there was no significant difference among the treatment groups. Flohr et al. [15] reported no difference in ADFI during the lactation period among groups with vitamin D<sub>3</sub> supplementation between 1,500 and 6,000 IU/kg of the complete diet. In addition, Shelton et al. [17] reported no difference in total or daily feed intake during the lactation period when sows were fed diets supplemented with vitamin E at four levels, i.e., 11, 22, 33, and 44 IU/kg of diet, during the gestation and lactation periods. On the other hand, Lauridsen et al. [14] reported greater ADFI in lactating sows when they were fed the lowest dose of vitamin D (200 IU/kg of diet). However, they could not draw conclusions from the results because number of lactation days and the total feed intake were affected by the form and dose of vitamin D. In agreement with the above results, the present study indicated that the ADFI of lactation sows decreased with increasing levels of vitamin supplementation.

Lauridsen et al. [14] reported that the body weight of sows at weaning was different between groups receiving dietary vitamin D doses of 200, 800, 1,400, and 2,000 IU/kg of diet. Flohr et al.

[15] reported that the vitamin D<sub>3</sub> level in the diet did not affect the body weight of lactating sows at weaning. However, Mahan [16] reported that sow weight loss during the period of lactation increased with increasing dietary vitamin E level. However, they could not explain the weight loss in the sows. Several studies suggested that obese individuals tend to have low serum levels of 25(OH)D<sub>3</sub> [18,19]. However, recent studies suggested that adipose tissue could be a direct target of vitamin D, and that the hormone could control the formation and function of adipose tissue [20,21]. Therefore, the low serum levels of 25(OH)D<sub>3</sub> in obese individuals may be due to greater isolation by adipose tissue [22,23].

Vitamin D can act through numerous non-genomic mechanisms such as protein expression, inflammation, oxidative stress, and cellular metabolism [24]. In addition, Ding et al. (2012) reported a signaling role of vitamin D in adipocytes, and discussed the potential mechanisms for vitamin D to affect tissue development and function. These findings suggested that vitamin D may have played a role in increasing the backfat thickness of the sows in the present study. Greater backfat thickness at farrowing is associated with lower lactation feed intake [25,26]. The effects of body fat content on voluntary lactation feed intake were reported to involve several mechanisms, including turnover of body fat tissue [27], insulin and leptin levels in blood [28], and milk production [29,30]. These mechanisms explain the reductions of feed intake and body weight during the lactation period observed in the present study.

**Reproductive performance of sows and litter performance**

The effects of different levels of vitamins in the gestation diet on the reproductive performance of sows and litter performance are presented in Table 5. There were no significant differences in the numbers of total born, born alive, or stillborn piglets among sows

**Table 4. Effects of vitamin supplementation levels in gestation diets on growth performance of sows during lactation**

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
Body weight (kg)							
24 hours postpartum	246.99	247.81	245.65	243.01	4.252	0.735	0.847
21 day of lactation	241.67	235.40	237.32	223.46	4.221	0.194	0.660
BW changes (0–21 d)	-5.32	-12.41	-10.91	-19.55	1.909	0.027	0.570
Backfat thickness (mm)							
24 hours postpartum	20.33	21.27	23.35	20.33	1.161	0.830	0.375
21 day of lactation	19.11	21.91	20.32	20.33	0.946	0.739	0.653
24 hours postpartum	-1.22	-0.64	-3.03	-0.00	0.503	0.881	0.197
ADFI (kg)	5.05	4.90	4.87	4.21	0.143	0.060	0.375
WEI (day)	4.81	5.89	5.07	4.67	0.189	0.447	0.047

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM) based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement. Lin., linear; Quad., quadratic; BW, body weight; ADFI, average daily feed intake; WEI, weaning to estrus interval.



**Table 5.** Effects of vitamin supplementation levels in gestation diets on reproductive performance of gestating sows

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
No. of piglets							
Total born	14.56	14.27	13.64	15.33	0.495	0.721	0.336
Born alive	12.00	13.45	12.09	13.56	0.435	0.423	0.995
Stillbirth	1.56	0.73	1.00	1.56	0.237	0.904	0.159
After cross-foster <sup>2)</sup>	12.11	11.84	11.66	12.00	0.116	0.626	0.160
21 day of lactation	10.67	10.36	10.09	10.78	0.263	0.981	0.368
Litter weight (kg)							
Total litter weight	20.20	19.44	18.60	19.98	0.601	0.794	0.394
Litter birth weight	17.92	18.65	17.06	18.34	0.584	0.953	0.818
After cross-foster <sup>2)</sup>	17.94	16.34	16.56	16.22	0.416	0.219	0.329
21 day of lactation	59.71	54.31	55.07	56.05	1.897	0.543	0.497
Litter weight gain	41.77	39.21	38.66	39.83	1.776	0.714	0.617
Piglet weight (kg)							
Piglet birth weight	1.49	1.41	1.43	1.36	0.027	0.205	0.855
After cross-foster <sup>2)</sup>	1.48	1.38	1.42	1.35	0.030	0.236	0.827
21 day of lactation	5.72	5.30	5.42	5.19	0.145	0.302	0.750
Piglet weight gain	4.24	3.92	4.00	3.84	0.144	0.427	0.783

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM) based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement.

<sup>2)</sup>After cross-fostering day within 24 hours postpartum.

Lin., linear; Quad., quadratic.

fed diets containing different vitamin levels. In addition, the different dietary vitamin levels showed no effects on total litter weight, litter birth weight, or litter weight at 21 days of lactation. Lauridsen et al. [14] reported that the number of stillborn piglets decreased with vitamin D<sub>3</sub> levels of 1,400 and 2,000 IU compared to 200 and 800 IU. They reported that the performance of the litter from birth to weaning was not influenced by the dietary vitamin D level, but the litter body weight after 2 weeks was increased by increasing the dose of vitamin D. Flohr et al. [15] reported that the levels of maternal vitamin D<sub>3</sub> did not affect litter size, mummies, stillbirths, number of piglets born alive, number of weaning pigs, or suckling pig performance. Similarly, no differences were observed in litter size, average weight, or total litter weight according to the level of vitamin E supplementation [17]. Several studies have indicated that the supplementation of the diet with 30–60 IU/kg of vitamin E is sufficient to optimize reproductive performance of pigs [16,31].

### Blood profiles

There were no significant differences in the serum levels of 25-(OH)-vitamin D or vitamin E in the blood of gestating sows according to the level of vitamin in the diet (Table 6). However, the serum 25(OH)D<sub>3</sub> concentration in sows at 90 days of gestation increased linearly with increasing dietary vitamin level (linear,  $p = 0.003$ ). Furthermore, the serum vitamin E level of sows during

gestation increased linearly with increasing dietary vitamin level ( $p < 0.05$ ). There were no significant differences in serum phosphorus concentrations among sows fed diets with different levels of vitamin supplementation (Table 7). On the other hand, the calcium concentration at 70 days of gestation was significantly elevated in sows fed a diet containing three times the NRC (2012)-recommended vitamin level. In addition, the calcium concentration tended to increase with increasing vitamin supplementation level at 70 and 110 days of gestation (linear,  $p = 0.067$ ,  $p = 0.077$ , respectively). The effects of vitamin supplementation level of the gestation diet on the blood profiles of sows and piglets during the lactation period are shown in Tables 8 and 9, respectively. Vitamin supplementation levels had no effect on the blood profiles of sows. However, significant differences were observed in the concentrations of 25-(OH)-vitamin D in piglets at 24 hours postpartum and day 21 of lactation ( $p < 0.05$ ). The concentration of 25-(OH)-vitamin D increased linearly in piglets at 21 days of lactation when the vitamin level was increased in the diet of sows (linear,  $p = 0.011$ ). Significant differences were observed in vitamin E levels according to the vitamin supplementation level. The concentration of vitamin E in the blood of sows fed the NRC (2012) vitamin requirement, i.e., 44 IU of vitamin E/kg of diet, was significantly lower than in the other treatment groups ( $p < 0.01$ ). In addition, the serum concentration of vitamin E in sows increased with increasing dietary

**Table 6.** Effects of vitamin supplementation levels in gestation diets on serum vitamin concentrations of sows during the gestation period

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
25-(OH) vitamin D (ng/mL)							
Initial	-----78.82-----						
14 days	102.0	85.6	103.6	102.4	4.81	0.669	0.454
28 days	123.0	96.6	124.3	133.0	5.97	0.255	0.129
70 days	125.3	137.0	142.7	150.0	5.02	0.109	0.832
90 days	106.0	115.9	149.7	157.1	7.11	0.003	0.596
110 days	134.3	133.5	136.3	166.2	3.75	0.136	0.476
Vitamin E (Tocopherol) (µmol/L)							
Initial	-----7.62-----						
14 days	4.3	3.7	4.4	4.3	0.23	0.719	0.616
28 days	8.4	8.5	12.2	13.0	0.81	0.012	0.783
70 days	7.9	9.8	11.7	10.9	0.92	0.236	0.511
90 days	12.2	13.9	18.9	20.2	1.35	0.015	0.945
110 days	9.6 <sup>B</sup>	12.7 <sup>A</sup>	13.8 <sup>A</sup>	14.6 <sup>A</sup>	0.97	0.013	0.297

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM)-based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement.

<sup>A,B</sup>Means with different capital letter superscripts in the same row are significantly different (*p* < 0.01).

Lin., linear; Quad., quadratic.

**Table 7.** Effects of vitamin supplementation levels in gestation diets on serum calcium and phosphorus level during the gestation period

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
Calcium (mg/dL)							
Initial	-----9.72-----						
14 days	9.9	9.6	10.0	10.0	0.09	0.349	0.348
28 days	9.8	9.9	10.0	10.0	0.08	0.451	0.671
70 days	9.8 <sup>ab</sup>	10.0 <sup>a</sup>	9.8 <sup>ab</sup>	9.5 <sup>b</sup>	0.06	0.067	0.019
90 days	9.3	9.4	9.1	9.6	0.09	0.479	0.240
110 days	9.4	9.6	9.4	10.1	0.11	0.077	0.391
Inorganic phosphorus (mg/dL)							
Initial	-----6.30-----						
14 days	6.0	6.1	5.8	6.2	0.14	0.914	0.629
28 days	6.2	6.5	6.3	6.9	0.14	0.112	0.612
70 days	5.9	5.9	6.1	5.7	0.10	0.756	0.330
90 days	5.6	5.7	5.9	5.6	0.17	0.985	0.589
110 days	7.3	6.6	6.7	7.7	0.18	0.269	0.020

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM) based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement.

<sup>a,b</sup>Means with different small letter superscripts in the same row are significantly different (*p* < 0.05).

Lin., linear; Quad., quadratic.

vitamin level. The serum 25(OH)D<sub>3</sub> level has been established as a functional indicator of vitamin D by the Food and Nutrition Board [32]. As expected based on a previous study in swine [33], plasma 25(OH)D<sub>3</sub> reflected the dietary dose of vitamin D. Lauridsen et al. [14] reported that the 25(OH)D<sub>3</sub> level in plasma increased linearly with increasing dietary dose of vitamin D. However, dietary

vitamin D treatment exerted no influence on the plasma concentrations of calcium or phosphorus in the gilts, whereas the calcium concentration in the plasma of piglets was influenced by the interaction between supplementation level and day of blood sampling. Zeni et al. [34] reported that the calcium demands during lactation are greater than those during pregnancy. Unfortunately, this experi-

**Table 8.** Effects of vitamin supplementation levels in gestation diets on blood profiles of sows in the lactation period

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
25-(OH) vitamin D (ng/mL)							
24 hours postpartum	143.0	131.4	152.4	164.4	7.61	0.377	0.239
21 days of lactation	138.75	120.4	137.8	152.8	5.59	0.185	0.207
Vitamin E (Tocopherol) (μmol/L)							
24 hours postpartum	3.5	5.4	6.5	5.6	0.52	0.141	0.133
21 days of lactation	7.4	5.7	5.0	6.2	0.43	0.313	0.088
Calcium (mg/dL)							
24 hours postpartum	9.4	10.0	10.0	9.7	0.16	0.592	0.185
21 days of lactation	10.1	10.2	9.7	10.1	0.11	0.654	0.372
Phosphorus (mg/dL)							
24 hours postpartum	8.6	6.2	7.8	7.4	0.36	0.689	0.237
21 days of lactation	6.2	6.3	6.0	6.1	0.14	0.693	0.920

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM) based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement.

Lin., linear; Quad., quadratic.

**Table 9.** Effects of vitamin supplementation levels in gestation diets on blood profiles of piglets in the lactation period

	Treatment <sup>1)</sup>				SEM	p-value	
	V1	V3	V6	V9		Lin.	Quad.
25-(OH) vitamin D (ng/mL)							
24 hours postpartum	40.3 <sup>b</sup>	30.1 <sup>b</sup>	31.8 <sup>b</sup>	141.5 <sup>a</sup>	11.35	0.063	0.062
21 days of lactation	18.1 <sup>b</sup>	15.5 <sup>b</sup>	16.4 <sup>b</sup>	30.2 <sup>a</sup>	2.02	0.011	0.022
Vitamin E (Tocopherol) (μmol/L)							
24 hours postpartum	4.5 <sup>B</sup>	12.4 <sup>A</sup>	12.8 <sup>A</sup>	12.4 <sup>A</sup>	1.11	0.009	0.017
21 days of lactation	12.8	10.8	9.6	11.4	0.72	0.499	0.188
Calcium (mg/dL)							
24 hours postpartum	10.6	10.1	9.7	10.0	0.16	0.238	0.190
21 days of lactation	11.0	11.4	10.9	11.0	0.12	0.519	0.876
Phosphorus (mg/dL)							
24 hours postpartum	5.7	8.0	5.4	6.5	0.40	0.838	0.674
21 days of lactation	9.6	9.6	9.5	9.5	0.24	0.817	0.967

<sup>1)</sup>Treatment: V1, corn-soybean meal (SBM)-based diet with 1× National Research Council (NRC, 2012) vitamin requirement; V3, corn-SBM-based diet with 3× NRC (2012) vitamin requirement; V6, corn-SBM-based diet with 6× NRC (2012) vitamin requirement; V9, corn-SBM-based diet with 9× NRC (2012) vitamin requirement.

<sup>A,B</sup>Means with different capital letter superscripts in the same row are significantly different ( $p < 0.01$ ).

<sup>a,b</sup>Means with different small letter superscripts in the same row are significantly different ( $p < 0.05$ ).

Lin., linear; Quad., quadratic.

ment were unable to analyze the concentration of vitamin D in the milk of sows, but the concentration of 25(OH)D<sub>3</sub> in cow milk is known to increase with cholecalciferol treatment before parturition [35]. Shelton et al. [17] reported that the plasma  $\alpha$ -tocopherol level in suckling pigs increased with increasing level of vitamin E in the diet of sows. Mahan et al. [16] reported increased  $\alpha$ -tocopherol concentrations in colostrum and milk with increasing levels of dietary vitamin E supplementation. As neonatal pigs are deficient in vitamin E, the concentration of  $\alpha$ -tocopherol increas-

es in pigs nursed by the sow [31], and decreases in postweaning [36].

## Conclusions

There were no significant differences in growth performance or reproductive performance during the gestation or lactation period, although the serum vitamin concentrations of piglets increased with increasing levels of vitamin supplementation in the diet of gestating sows. In conclusion, the current NRC (2012) vitamin



requirement is sufficient for gestating sows, and additional vitamin supplementation of the diet did not show any beneficial effects for gestating and lactating sows.

### Competing interests

We certify that there are no conflicts of interest regarding the material presented in this manuscript.

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### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

### Authors' contributions

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### Ethics approval and consent to participate

All experimental procedures involving animals were conducted in accordance with the Animal Experimental Guidelines of the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC; SNU-160819-11).

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