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Administration of vitamin D₃ by injection or drinking water alters serum 25-hydroxycholecalciferol concentrations of nursery pigs

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Objective: Two experiments were conducted to evaluate vitamin D₃ administration to nursery pigs by injection or in drinking water on serum 25-hydroxycholecalciferol (25-OHD₃) concentrations.
Methods: At weaning, 51 pigs (27 and 24 pigs in experiments 1 and 2, respectively) were allotted to vitamin D₃ treatments. Treatments in experiment 1 were: i) control (CON), no vitamin administration beyond that in the diet, ii) intramuscular (IM) injection of 40,000 IU of vitamin D₃ at weaning, and iii) water administration, 5,493 IU of vitamin D₃/L drinking water for 14 d post-weaning. Treatments in experiment 2 were: i) control (CON), no vitamin administration, and ii) water administration, 92 IU of d-α-tocopherol and 5,493 IU of vitamin D₃/L drinking water for 28 d postweaning. The lightest 2 pigs within each pen were IM injected with an additional 1,000 IU of d-α-tocopherol, 100,000 IU of retinyl palmitate, and 100,000 IU of vitamin D₃.
Results: In both experiments, serum 25-OHD₃ was changed after vitamin D₃ administration (p<0.05). In experiment 1, injection and water groups had greater values than CON group through d 35 and 21 post-administration, respectively (p<0.05). In experiment 2, serum values peaked at d 3 post-administration in the injection groups regardless of water treatments (p<0.05) whereas CON and water-only groups had peaks at d 14 and 28 post-administration, respectively (p<0.05). Even though the injection groups had greater serum 25-OHD₃ concentrations than the non-injection groups through d 7 post-administration regardless of water treatments (p<0.05), the water-only group had greater values than the injection-only group from d 21 post-administration onward (p<0.05).
Conclusion: Serum 25-OHD₃ concentrations in pigs increased either by vitamin D₃ injection or drinking water administration. Although a single vitamin D₃ injection enhanced serum 25-OHD₃ concentrations greater than water administration in the initial period post-administration, a continuous supply of vitamin D₃ via drinking water could maintain higher serum values than the single injection.

Keywords: Drinking Water; Injection; Vitamin D₃; Swine

INTRODUCTION

Vitamin D is important for not only Ca and P homeostasis and bone integrity but also immunity in pigs [1]. Recently, vitamin D has been an issue in pig nutrition because of purported vitamin D deficiency on swine farms [2]. Piglets are born with low plasma vitamin D levels [3] and confined housing allows limited vitamin D synthesis in suckling and nursery pigs [4] because of the inaccessibility of the pigs to sunlight. Even though sow colostrum and milk contribute to an increase of plasma vitamin D concentrations during the suckling period [5-7], plasma vitamin D status in the nursery period is still lower under confinement housing than outdoor housing [4], which raises the question whether an additional supply of vitamin D might be needed for nursery pigs to maintain their normal growth and bone development.

Recently, several methods have been investigated to enhance plasma vitamin D status of pigs in which vitamin D_3 administration to sows and suckling pigs by dietary supplementation, oral administration or IM injection increased plasma 25-hydroxycholecalciferol (25-OHD$_3$) levels of newborn and suckling pigs [5-9]. Furthermore, Petersen et al [10] reported that oral vitamin D_3 administration to pigs at birth and weaning under PRRS virus inoculation at weaning increased the level of antibodies for PRRS virus indicating that vitamin D_3 supplementation has a potentially positive effect on the immune response to certain disease conditions. However, there is limited information about vitamin D administration effects on nursery pigs and their differences among administration routes. Jang et al [5] reported that vitamin D_3 supplementation in drinking water of weanling pigs for 14 d increased plasma 25-OHD$_3$ concentrations at d 14 postweaning. Similarly, Flohr et al [8] reported that vitamin D_3 supplementation to nursery pigs via drinking water for 10 d from weaning increased serum 25-OHD$_3$ concentrations at d 10 postweaning. Additionally, fat-soluble vitamin supplementation (vitamin D_3 and E) to newborn pigs orally or by injection has been demonstrated to increase plasma vitamin status of pigs [5]. In spite of positive effects of vitamin D_3 and E administration in improving plasma vitamin status of newborn and nursery pigs, previous studies lack investigation on temporal change of plasma vitamin levels and their potential interaction with administration routes. Therefore, the objective of this study was to evaluate the effect of vitamin D_3 administration to nursery pigs by injection or in drinking water on temporal plasma vitamin status when pigs were under an immune challenge or not.

MATERIALS AND METHODS

All procedures in this study were conducted in accordance with guidelines stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching by the Federation of Animal Science Societies [11].

Animals and experimental design

In experiment 1, a total of 27 crossbred pigs [Yorkshire×Duroc, (Yorkshire×Landrace)×Duroc; average weaning age: 19.9±4 d] were used. At weaning, pigs were allotted into 3 treatments in 3 replicates with 3 pigs per pen (2 barrows and 1 gilt) based on sex and body weight (BW) in a randomized complete block design. Treatments were: i) control (CON): no supplemental vitamin D_3 beyond that supplied in the diet, ii) intramuscular (IM) injection of 40,000 IU of vitamin D_3 as used in Jang et al [5,6] for newborn pigs and iii) water administration of 5,493 IU of vitamin D_3 per L of drinking water during 14 d postweaning. The commercial products used were from Stuart Products Inc. (Bedford, TX, USA). Daily vitamin D_3 intake via drinking water was estimated using the equation reported by Brooks et al [12] adopted by NRC [13]; water intake of weaning piglet (L/d) = 0.149+(3.053×daily dry feed intake, kg/d) with an estimated feed intake value of 7 to 11 kg BW [13], and dosage of vitamin D_3 in drinking water (5,493 IU/L). The total estimated vitamin D_3 intake for 14 d was 121,418 IU which was approximately 3 times the vitamin D_3 provision via IM injection. All pigs were challenged with 4 mg of ovalbumin with Freund's Incomplete Adjuvant per mL at weaning and d 14 postweaning as an immune stimulant. From d 14 postweaning, all pigs were provided water with no supplemental vitamins.

In experiment 2, a total of 24 crossbred pigs [Yorkshire×Duroc, (Yorkshire×Landrace)×Duroc; average weaning age: 19.9±1 d] were used. At weaning, pigs were allotted into 2 treatments in 3 replicates with 4 pigs per pen (2 barrows and 2 gilts) based on sex and BW in a randomized complete block design. Treatments were: i) control (CON): no supplemental vitamin D_3, and ii) water administration of 92 IU of d-α-tocopherol and 5,493 IU of vitamin D_3 (the same dosage as experiment 1) per L of drinking water during 28 d postweaning. The products used were from Stuart Products Inc. (Bedford, TX, USA). Then, the lightest 2 pigs (1 barrow and 1 gilt) within a pen were injected with 2 mL of 500 IU of vitamin E as d-α-tocopherol, 50,000 IU of vitamin A as retinyl palmitate, and 50,000 IU of vitamin D_3/mL at weaning using a commercial product at recommended levels to investigate whether additional vitamin administration might lead to improving growth performance comparable to heavy pigs within the pen. From d 28 postweaning, all pigs were provided water with no supplemental vitamins.

Diets, housing, and vitamin administration

The diets for experiments 1 and 2 were formulated to meet or exceed NRC [14] nutrient requirement estimates. In experiment 1, corn-soybean meal-based starter diets were provided for 2 phases feeding (Phase 1, d 0 to 14 postweaning; Phase 2, d 14 to 35 postweaning). Phase 1 and 2 diets contained 1.20% and 1.01% standardized ileal digestible lysine with the following per kg diet: 9,007 IU vitamin A, 2,253 IU vitamin D_3, and 60 IU vitamin E (Akey A Sow VTM premix Se Yeast; Provimi North America, Inc., Brookville, OH, USA). In experiment 2, corn-SBM-based starter diets were provided for 2 phase feeding (Phase 1, d 0 to 14 postweaning; Phase 2, d 14 to 35 postweaning). Phase 1 and 2 diets contained 1.21% and 1.02% standardized ileal digestible lysine with the following per kg diet: 11,000 IU of vitamin A, 1,100 IU of vitamin D_3, and 77 IU of vitamin E. All pigs were housed in $1.22×1.22$ m^2 raised-deck nursery pens with plastic coated expanded metal flooring in an environmentally controlled nursery facility without windows. Pigs were provided the diet *ad libitum* and water was freely available from a water nipple throughout the experimental period. Injectable products were provided to each pig in the trapezius muscle. For the drinking water administration, 14.06 mL of vitamin D_3 product (50,000 IU vitamin D_3/mL) in experiment 1 or 23.44 mL of vitamin D_3 product (30,000 IU vitamin D_3, and 500 IU d-α-tocopherol/mL) in experiment 2 was

added into a 1 L stock solution, thoroughly mixed and then the stock solution containing vitamins was metered at a rate of 1:128.

Data and sample collection

Even though growth performance was not a primary response objective for vitamin D_3 administration in the current study, BW and feed disappearance were recorded weekly (including at d 3 and 31 postweaning in experiment 2) to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). In experiment 1, blood samples were collected from the jugular vein at d 0 (before administration of any treatments), 1, 2, 3, 7, 14, 17, 21, 28, and 35 postweaning, and in experiment 2, blood samples were collected from the jugular vein at d 0 (before administration of any treatments), 1, 2, 3, 7, 14, 21, 28, 31, and 35 postweaning. Blood samples were centrifuged at 1,700 g for 15 minutes at 4°C; serum samples were then aliquoted into microtubes and stored at –20°C until analysis. Serum samples from individual pigs were pooled within a pen (experiment 1) and within a pen and injection treatment across sex (experiment 2) before analysis. Serum samples were sent to the Heartland Assay, LLC. (Ames IA, USA) for $25\text{-}OHD_3$ assay and the Iowa State University Veterinary Diagnostic Laboratory (Ames IA, USA) for α-tocopherol assay.

Statistical analysis

In experiment 1, all data analyses were conducted by repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) for a randomized complete block design. Model terms were the effects of treatment, day, and treatment×day interaction as fixed effects and replicate as a random effect with a heterogeneous autoregressive [ARH(1)] covariance structure. Least square mean separations were conducted using the PDIFF option in SAS when there was an interaction between day and treatment. In experiment 2, all data analyses were conducted by repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., USA) for a randomized complete block design. Model terms were the effects of water, injection, day, and interactions of water× injection, water×day, injection×day and water×injection×day as fixed effects and replicate as a random effect with a heterogeneous autoregressive [ARH(1)] covariance structure. Least square mean separations were conducted using the PDIFF option in SAS where there were interactions of water×injection, water×day, injection× day, and water×injection×day. To avoid potential bias because of differing standard error of the mean (SEM) within the injection and non-injection groups, statistical analysis within each injection treatment was performed. Individual pen was considered as experimental unit. Statistical differences were considered significant at p<0.05, and a trend at p<0.10.

RESULTS AND DISCUSSION

Weaning is perhaps the largest stress in a pig's life due to social,

environmental, and dietary changes which can cause diarrhea, growth retardation and high susceptibility to peri- and post-weaning diseases. Recently, it has been reported that low serum vitamin D concentrations and vitamin D deficiency are related to porcine wasting-catabolic diseases such as periweaning failure to thrive syndrome and bone metabolic diseases (e.g., kyphosis, rickets) [15,16]. Even though typical nursery diets, such as reported in an industry survey [17], often contain as much as 10 times greater amount of vitamin D from ingredients and vitamin premixes compared to NRC requirement estimates [13], serum $25\text{-}OHD_3$ concentrations of nursery pigs are lower than expected due to confinement housing and insufficient consumption of vitamin D via maternal milk [4]. Therefore, this study evaluated the effect of vitamin D_3 administration to nursery pigs via IM injection, water supply, or their combination to improve serum vitamin D status of nursery pigs.

Growth performance

In experiment 1, there were no differences in BW, ADG, ADFI, and G:F throughout the experimental periods (p>0.52; Table 1) even though all pigs were injected with ovalbumin as a means to stimulate the immune system which indicates that vitamin D_3 administration had no specific benefit to ameliorate immune stress of weaning pigs in this experiment. In experiment 2, even though there were apparent differences in BW for the entire periods (p<0.05) and in ADG at d 0 to 14 (p = 0.09), d 28 to 35 (p< 0.05), and d 0 to 35 (p = 0.08) between non-injection and injec-

Table 1. Growth performance of pigs administered with vitamin D_3 by injection or in drinking water under ovalbumin challenge (experiment 1)[1)-3)]

Item	Treatment[4)]			SEM	p-value
	Control	Injection	Water		
BW (kg)					
d 0	6.65	6.60	6.70	0.60	0.91
d 14	11.06	11.15	11.23	0.64	0.83
d 35	24.13	24.24	24.34	1.34	0.99
ADG (kg/d)					
d 0-14	0.315	0.325	0.324	0.01	0.82
d 14-35	0.622	0.624	0.624	0.04	1.00
d 0-35	0.499	0.504	0.504	0.02	0.98
ADFI (kg/d)					
d 0-14	0.499	0.502	0.510	0.02	0.90
d 14-35	1.153	1.094	1.127	0.04	0.52
d 0-35	0.891	0.857	0.881	0.02	0.64
G:F					
d 0-14	0.634	0.647	0.637	0.03	0.95
d 14-35	0.540	0.570	0.553	0.02	0.62
d 0-35	0.561	0.588	0.572	0.02	0.73

SEM, standard error of the mean; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio; FIA, Freund's incomplete adjuvant.
[1)] Values are least squares means (n = 3 per treatment).
[2)] All pigs were challenged with 4 mg of ovalbumin with FIA per mL at weaning and d 14 postweaning.
[3)] Water administration was stopped at d 14 postweaning.
[4)] See text for treatment description.

tion groups, it was due to the difference in initial BW between those groups (Table 2). However, there was no difference in BW and ADG when initial BW was considered as a covariate (p>0.51). Average daily feed intake and G:F could be calculated only on a pen basis (water treatment) because housing was not separated for the injection treatments within the water treatments. In the examination of performance pooled across the injection treatments, there were no differences in BW, ADG, ADFI, and G:F except for a greater ADFI observed in the water treatments than the CON treatments from d 14 to 28 postweaning (p<0.05; Table 3). Based on the results of growth performance in both experiments 1 and 2, an additional administration of vitamin D_3 by IM injection and in drinking water had no beneficial effect on growth rate and feed efficiency regardless of the immune challenge by ovalbumin injection. These results agrees with previous studies that reported no influences of vitamin D_3 administration on pre- and postweaning growth performance [5,7,8,18] although it should be noted that the current study had low number of observations (n = 3) for growth response assessment.

Serum 25-OHD$_3$ concentrations
In experiment 1, treatment and day effects, and an interaction between treatment and day were observed (p<0.01; Table 4) on serum 25-OHD$_3$ concentrations. Serum 25-OHD$_3$ concentrations

of the pigs in the injection group were always greater than the CON and water groups through d 35 post-administration (p<0.05). Serum 25-OHD$_3$ concentrations of the pigs in the water group were greater than those in the CON group until d 21 post-administration (p<0.05) and similar at d 28 and 35 of post-administration. This result agrees with Jang et al [6] who reported that plasma 25-OHD$_3$ concentrations of suckling piglets increased with vitamin D_3 administration either orally or by IM injection but the injection had greater efficiency to enhance plasma 25-OHD$_3$ concentrations compared to oral administration. Jang et al [5] also reported that vitamin D_3 administration to nursery pigs via drinking water from weaning for 14 d increased plasma 25-OHD$_3$ concentrations at d 14 postweaning.

In the temporal change of serum 25-OHD$_3$ concentrations (Table 4), serum values in the injection group reached a peak at d 3 post administration and were reduced afterward which is similar to that reported by Jang et al [6]. However, this peak day was postponed a day later than from Jang et al [6] that reported the peak day at d 2 after a single vitamin D_3 injection to newborn pigs with the same amount of vitamin D_3 as used in the current study. It has been reported that when dairy calves received radioactive-labeled vitamin D_3 orally, plasma levels of labeled vitamin D_3 had a predominant peak between 1 to 2 d, while plasma levels of labeled 25-OHD$_3$ became predominant with its maximum con-

Table 2. Growth performance of pigs with vitamin D_3 and E administration in drinking water with or without vitamin A, D_3, E injection (experiment 2)[1)-3)]

Item	Non-injection		Injection		SEM	p-value		
	Control	Water	Control	Water		Water	Injection	Water×Injection
BW (kg)								
d 0	6.50	6.48	5.66	5.57	0.32	0.80	0.004	0.88
d 14	10.32	10.50	8.91	8.50	0.60	0.80	0.008	0.53
d 28	17.05	18.10	14.83	15.01	1.36	0.56	0.036	0.67
d 35	22.72	23.52	19.56	19.92	1.78	0.65	0.031	0.86
ADG (kg/d)								
d 0-14	0.273	0.287	0.232	0.209	0.030	0.88	0.09	0.55
d 14-28	0.480	0.543	0.423	0.465	0.057	0.27	0.17	0.82
d 28-35	0.811	0.774	0.676	0.701	0.065	0.89	0.04	0.47
d 14-35	0.591	0.620	0.507	0.544	0.059	0.45	0.10	0.93
d 0-35	0.463	0.487	0.397	0.410	0.045	0.61	0.08	0.88
BW with initial BW as a covariate (kg)[4)]								
d 0	6.05	6.05	6.05	6.05	-	-	-	-
d 14	9.69	9.90	9.47	9.18	0.47	0.93	0.46	0.58
d 28	15.63	16.76	16.08	16.52	1.11	0.47	0.95	0.74
d 35	20.88	21.76	21.19	21.89	1.44	0.57	0.91	0.95
ADG with initial BW as a covariate (kg/d)[4)]								
d 0-14	0.260	0.274	0.244	0.223	0.033	0.93	0.46	0.58
d 14-28	0.425	0.490	0.472	0.524	0.049	0.24	0.54	0.89
d 28-35	0.755	0.720	0.725	0.762	0.059	0.99	0.94	0.51
d 14-35	0.533	0.565	0.558	0.605	0.050	0.42	0.62	0.88
d 0-35	0.424	0.449	0.433	0.453	0.041	0.57	0.91	0.95

SEM, standard error of the mean; BW, body weight; ADG, average daily gain.
[1)] Values are least squares means (n = 3 per treatment).
[2)] See text for treatment description.
[3)] Water administration was stopped at d 28 postweaning.
[4)] Because there was a significant difference in initial BW between injection and non-injection groups, initial BW was considered as a covariate.

Table 3. Growth performance of pigs with vitamin D_3 and E administration in drinking water with or without vitamin A, D_3, E injection (experiment 2)[1)-3)]

Item	Control	Water	SEM	p-value
BW (kg)				
d 0	6.08	6.01	0.26	0.21
d 14	9.62	9.45	0.53	0.80
d 28	15.94	16.46	1.26	0.75
d 35	21.14	21.59	1.66	0.83
ADG (kg/d)				
d 0-14	0.253	0.246	0.025	0.86
d 14-28	0.452	0.501	0.053	0.53
d 28-35	0.743	0.733	0.061	0.87
d 14-35	0.549	0.578	0.055	0.68
d 0-35	0.430	0.445	0.042	0.80
ADFI (kg/d)				
d 0-14	0.495	0.434	0.029	0.27
d 14-28	0.782	0.831	0.046	0.03
d 28-35	1.294	1.260	0.090	0.82
d 14-35	0.952	0.974	0.052	0.72
d 0-35	0.769	0.758	0.035	0.81
G:F				
d 0-14	0.506	0.570	0.043	0.39
d 14-28	0.573	0.603	0.044	0.68
d 28-35	0.574	0.582	0.025	0.76
d 14-35	0.574	0.591	0.025	0.64
d 0-35	0.556	0.585	0.029	0.52

SEM, standard error of the mean; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio.
[1)] Values are least squares means (n = 3 per treatment).
[2)] See text for treatment description.
[3)] Water administration was stopped at d 28 postweaning.

Table 4. Time-dependent serum 25-OHD$_3$ concentration changes of weaning pigs with vitamin D_3 administration by injection or in drinking water under ovalbumin challenge (experiment 1)[1)-3)]

Item	Treatment (ng/mL)[4)]			SEM	p-value
	Control	Injection	Water		
Day[5)]					
0	6.5	6.8	7.0	0.8	0.90
1	5.8[c]	58.3[a]	11.3[b]	1.4	<0.0001
2	5.9[c]	70.3[a]	19.5[b]	1.8	<0.0001
3	7.3[c]	79.1[a]	30.3[b]	1.6	<0.0001
7	21.5[c]	73.6[a]	45.9[b]	1.7	<0.0001
14	36.0[c]	60.2[a]	46.0[b]	2.2	<0.0001
17	29.1[c]	52.5[a]	39.0[b]	1.9	<0.0001
21	27.2[c]	44.6[a]	31.2[b]	1.3	<0.0001
28	28.6[b]	38.8[a]	25.6[b]	1.9	<0.0001
35	28.9[b]	37.6[a]	30.2[b]	2.2	0.01

25-OHD$_3$, 25-hydroxycholecalciferol; SEM, standard error of the mean; FIA, Freund's incomplete adjuvant.
[1)] Values are least squares means (n = 3 per treatment).
[2)] All pigs were challenged with 4 mg of ovalbumin with FIA per mL at weaning and d 14 postweaning.
[3)] Repeated measures was used for data analysis (treatment, day effects, and day × treatment interaction, p<0.01).
[4)] See text for treatment description.
[5)] Day after vitamin administration except d 0 is before administration. Water administration was stopped at d 14 postweaning.
[a-c] Means within the same row without a common superscript differ (p<0.05).

centrations between 2 to 4 d [19] which indicate that if vitamin D_3 was administered to the animal, it may require a period of time to be absorbed into the body and converted to the metabolic or circulating form of vitamin D_3 which is 25-OHD$_3$. However, in the current study, the only difference from Jang et al [6] was initial BW and age when the vitamin D_3 was administered which may explain the delayed peak day and the lower maximum 25-OHD$_3$ concentrations (3 times less than those in Jang et al [6]). Additionally, it should be stated that even though serum 25-OHD$_3$ concentrations increased with age or BW [4], the response to vitamin D_3 administration may differ by body size and there could be a dilution effect with increasing BW of pigs as suggested by Jang et al [5].

Regarding temporal changes of serum 25-OHD$_3$ concentrations in the CON and water groups (Table 4), serum values in those 2 groups increased up to d 14 postweaning. However, serum 25-OHD$_3$ values in the CON group were maintained relatively constant from d 17 post-administration whereas a continuous reduction occurred in the water group from d 14 post-administration once vitamin D supply in drinking water was discontinued. This result agrees with Flohr et al [8] who reported that vitamin D_3 administration in drinking water for 10 d postweaning increased serum 25-OHD$_3$ concentrations at d 10 post-administration, and

then the serum values decreased. West et al [20] also reported that vitamin D_3 administration in drinking water to nursery pigs for 5 d increased serum 25-OHD$_3$ concentrations at d 5 post-administration with peaks and a rapid reduction started once vitamin D_3 supply was discontinued.

Experiment 2 was a series study to investigate the effect of vitamin D_3 and E administration in drinking water together with the injection of vitamin A, D_3, and E, and allowed the examination of the combined effect of vitamin administration between IM injection and drinking water administration associated with a longer period of water administration than experiment 1. In experiment 2 (Table 5), the water, injection treatment and day effects and all interactions between them on serum 25-OHD$_3$ concentrations were observed (p<0.05; p = 0.09 for interaction between the water and injection treatments) in which either water administration or injection of vitamin D_3 to the nursery pigs increased serum 25-OHD$_3$ concentrations as experiment 1 which again agrees with Flohr et al [8] and Jang et al [5,6]. Interestingly, pigs in the injection groups had greater serum 25-OHD$_3$ concentrations than those in the non-injection groups until d 7 post-administration regardless of water treatments (p<0.05) whereas the water-only group had similar values at d 14 post-administration and greater values compared with the injection-only group from d 21 post-administration onward (p<0.05); the water-only group was similar to the injection-water group from d 28 post-administration. However, there were different patterns in temporal changes of serum 25-OHD$_3$ concentrations by water treatment

Table 5. Time-dependent serum 25-OHD$_3$ concentration changes of weaning pigs with vitamin D$_3$ and E administration in drinking water with or without vitamin A, D$_3$, E injection (experiment 2)[1)-3)]

Item	Non-injection		Injection		SEM	p-value		
	Control	Water	Control	Water		Water	Injection	Water×injection
Day[4)]	-- ng/mL --							
0	11.81	11.41	11.26	12.27	1.42	0.83	0.91	0.96
1	11.58[b]	15.17[b]	120.20[a]	131.92[a]	7.81	0.33	<0.0001	<0.0001
2	12.66[d]	21.52[c]	141.52[b]	151.43[a]	2.33	0.0001	<0.0001	<0.0001
3	14.44[d]	29.23[c]	146.63[b]	154.39[a]	2.74	0.0001	<0.0001	<0.0001
7	27.87[c]	63.88[b]	124.01[a]	133.76[a]	4.62	<0.0001	<0.0001	<0.0001
14	35.74[c]	78.40[b]	89.74[b]	112.66[a]	4.67	<0.0001	<0.0001	<0.0001
21	31.29[d]	76.41[b]	64.48[c]	92.50[a]	2.45	<0.0001	<0.0001	<0.0001
28	29.27[c]	79.38[a]	48.88[b]	85.35[a]	2.78	<0.0001	<0.0001	<0.0001
31	31.23[c]	67.24[a]	46.75[b]	75.29[a]	3.46	<0.0001	0.001	<0.0001
35	34.71[c]	58.35[a]	48.01[b]	59.37[a]	3.51	<0.0001	0.045	<0.0001

25-OHD$_3$, 25-hydroxycholecalciferol; SEM, standard error of the mean.
[1)] Values are least squares means (n = 3 per treatment).
[2)] See text for treatment description.
[3)] Repeated measures was used for data analysis (water, injection and day effects, water×day, injection×day, and water×injection×day interactions, p<0.01; water×injection interaction, p = 0.09).
[4)] Day after vitamin administration except d 0 is before administration. Water administration was stopped at d 28 postweaning.
[a-d] Means within the same row without a common superscript differ (p<0.05).

between the non-injection and injection groups. Within the injection groups (Table 6), water administration (injection-water) increased serum 25-OHD$_3$ concentrations greater than the injection-only group at d 2 and from d 14 to 35 post-administration (p<0.05) with numerical increases at d 1, 3, and 7 post-administration whereas within the non-injection groups, the pigs in the water-only group had greater serum 25-OHD$_3$ concentrations than those in the CON group during the entire period (p<0.01). This difference in the statistical analysis was due to a greater SEM in the injection group during d 1 to 7 and means that the water treatment effect was hidden by the injection treatment in the early period of the administration. Additionally, the increment of

serum 25-OHD$_3$ concentrations by drinking water administration of vitamin D$_3$ was greater within the non-injection pigs compared to the injection pigs from d 3 post-administration which illustrates the effect of vitamin D$_3$ administration in drinking water was more pronounced when it was a single route of administration.

In temporal changes of serum 25-OHD$_3$ concentrations (Table 5), serum values in the CON group peaked at d 14 postweaning and were maintained relatively constant thereafter whereas in the water-only group, serum values peaked at d 28 post-administration, and then decreased which demonstrates that serum values decline when vitamin D$_3$ administration in drinking water is

Table 6. Time-dependent serum 25-OHD$_3$ concentration changes of weaning pigs with vitamin D$_3$ and E administration in drinking water within vitamin A, D$_3$, E injection (experiment 2)[1)-3)]

Item	Non-injection		SEM	p-value	Injection		SEM	p-value
	Control	Water			Control	Water		
Day[4)]	-------------------- ng/mL ----------------				-------------------- ng/mL ----------------			
0	11.81	11.41	1.36	0.84	11.26	12.27	1.23	0.56
1	11.58	15.17	0.73	0.001	120.20	131.92	12.51	0.51
2	12.66	21.52	1.14	<0.0001	141.52	151.43	3.15	0.03
3	14.44	29.23	1.02	<0.0001	146.63	154.39	3.94	0.17
7	27.87	63.88	2.17	<0.0001	124.01	133.76	6.25	0.28
14	35.74	78.40	4.81	<0.0001	89.74	112.66	4.65	0.001
21	31.29	76.41	1.83	<0.0001	64.48	92.50	3.02	<0.0001
28	29.27	79.38	2.25	<0.0001	48.88	85.35	3.38	<0.0001
31	31.23	67.24	2.78	<0.0001	46.75	75.29	4.17	<0.0001
35	34.71	58.35	3.26	<0.0001	48.01	59.37	3.92	0.05

25-OHD$_3$, 25-hydroxycholecalciferol; SEM, standard error of the mean.
[1)] Values are least squares means (n = 3 per treatment).
[2)] See text for treatment description.
[3)] Repeated measures was used for data analysis (water, day effects, and water×day interaction within the non-injection or injection treatment, p<0.05).
[4)] Day after vitamin administration except d 0 is before administration. Water administration was stopped at d 28 postweaning.

discontinued as experiment 1. However, serum 25-OHD$_3$ concentrations of the injection groups peaked at d 3 post administration, and then reduced afterward regardless of water treatments as experiment 1 which again agrees with Jang et al [6]. Interestingly, the decrement was greater in the injection-only group compared with the injection-water group resulting in no differences in serum 25-OHD$_3$ concentrations between the water-only and injection-water groups from d 28 post-administration which were greater than the injection-only group. This result means that although a single vitamin D$_3$ injection enhanced serum 25-OHD$_3$ concentrations greater than its administration via drinking water, a continuous administration of vitamin D$_3$ via drinking water was more effective to maintain serum 25-OHD$_3$ concentrations relatively high regardless of additional vitamin D$_3$ supply from another source such as injection.

Comparing experiments 1 and 2, baseline values of serum 25-OHD$_3$ concentrations were greater in experiment 2 compared with experiment 1 whereas the temporal change of serum 25-OHD$_3$ concentrations was not different in the CON treatments between those experiments. It is obvious that the injection-only treatment in experiment 2 had 1.85 to 2.06 times greater serum 25-OHD$_3$ concentrations than the injection treatment in experiment 1 during the first 3 d post-administration due to 2.5 times greater amount of vitamin D$_3$ injection which agrees with Jang et al [5] who reported the greater plasma 25-OHD$_3$ concentrations at d 10 post-administration when the pigs were injected with higher amount of vitamin D$_3$ at birth. However, even though the amount of vitamin D$_3$ administration in drinking water was the same between the 2 experiments and the resultant serum 25-OHD$_3$ concentrations were similar between the 2 experiments at d 2 and 3 post-administration, the water-only treatment in experiment 2 had 1.4 to 1.7 times greater serum values than the water treatment in experiment 1 from d 7 to 14 post-administration.

Serum α-tocopherol concentrations

In experiment 2, injection and day effects, and interactions between water and day and between injection and day on serum α-tocopherol concentrations were observed (p<0.01; Table 7). Pigs in the injection groups had greater serum α-tocopherol concentrations than those in the non-injection groups until d 21 post-administration. Even though there were significant increases in serum α-tocopherol concentrations by water administration from d 7 to 28 post-administration (p<0.05; d 14, p = 0.102), an overall water effect was not observed. However, there might be heterogeneity of variance between the individual treatments due to much greater plasma α-tocopherol concentrations in the injection groups at d 1 to 3 post-administration. Therefore, a further analysis was conducted to detect the water treatment effect within the non-injection and injection treatments. Within the injection groups (Table 8), serum α-tocopherol concentrations were not different between the injection-water and injection-only groups except at d 21 and 28 post-administration on which the injection-water group had greater serum values (p<0.05) compared with the injection-only group. However, within the non-injection groups, the water-only group had greater serum α-tocopherol concentrations than the CON group from d 2 to 28 post-administration (p<0.05; p = 0.08 at d 3 post-administration). This result agrees with Amazan et al [21] who reported that vitamin E supplementation in drinking water for weaning pigs increased serum α-tocopherol concentrations even though there was a reduction of serum α-tocopherol concentration during the first 5 d post-weaning.

Table 7. Time-dependent serum α-tocopherol concentration changes of weaning pigs with vitamin D$_3$ and E administration in drinking water with or without vitamin A, D$_3$, E injection (experiment 2)[1)-3)]

Item	Non-injection		Injection		SEM	p-value		
	Control	Water	Control	Water		Water	Injection	Water×injection
Day[4)]	---------------------------------- μg/mL ----------------------------------							
0	4.61[ab]	4.14[b]	5.02[ab]	5.56[a]	0.36	0.92	0.013	0.04
1	4.53[b]	5.47[b]	378.37[a]	376.88[a]	27.62	0.99	<0.0001	<0.0001
2	3.51[b]	4.87[b]	81.47[a]	82.93[a]	7.21	0.85	<0.0001	<0.0001
3	2.46[b]	3.15[b]	19.68[a]	30.02[a]	4.57	0.23	<0.0001	<0.0001
7	1.24[c]	2.23[b]	2.56[b]	3.49[a]	0.30	0.002	<0.0001	<0.0001
14	0.89[c]	1.57[ab]	1.90[a]	2.09[a]	0.27	0.102	0.005	0.011
21	0.86[c]	1.94[a]	1.49[b]	2.04[a]	0.15	<0.0001	0.02	<0.0001
28	0.88[b]	2.32[a]	1.33[b]	2.27[a]	0.19	<0.0001	0.29	<0.0001
31	1.03	1.35	1.57	1.58	0.20	0.41	0.06	0.18
35	1.16	1.42	1.58	1.39	0.17	0.81	0.24	0.38

SEM, standard error of the mean.
[1)] Values are least squares means (n = 3 per treatment).
[2)] See text for treatment description.
[3)] Repeated measures was used for data analysis (injection, day effects, water×day and injection×day interactions, p<0.01; no water effect, water×injection and water×injection×day interactions).
[4)] Day after vitamin administration except d 0 is before administration. Water administration was stopped at d 28 postweaning.
[a-c] Means within the same row without a common superscript differ (p<0.05).

Table 8. Time-dependent serum α-tocopherol concentration changes of weaning pigs with vitamin D_3 and E administration in drinking water within vitamin A, D_3, E injection (experiment 2)[1-3]

Item	Non-injection		SEM	p-value	Injection		SEM	p-value
	Control	Water			Control	Water		
Day[4]	----------------- μg/mL ---------------				----------------- μg/mL ----------------			
0	4.61	4.14	0.38	0.38	5.02	5.56	0.30	0.21
1	4.53	5.47	0.60	0.27	378.37	376.88	44.20	0.98
2	3.51	4.87	0.47	0.046	81.47	82.93	10.86	0.92
3	2.46	3.15	0.27	0.08	19.68	30.02	6.88	0.29
7	1.24	2.23	0.10	<0.0001	2.56	3.49	0.42	0.13
14	0.89	1.57	0.23	0.047	1.90	2.09	0.27	0.61
21	0.86	1.94	0.15	<0.0001	1.49	2.04	0.13	0.004
28	0.88	2.32	0.15	<0.0001	1.33	2.27	0.19	0.001
31	1.03	1.35	0.18	0.22	1.57	1.58	0.18	0.97
35	1.16	1.42	0.16	0.27	1.58	1.39	0.17	0.46

SEM, standard error of the mean.
[1] Values are least squares means (n = 3 per treatment).
[2] See text for treatment description.
[3] Repeated measures was used for data analysis (water, day effects, and water × day interaction within the non-injection treatment, p < 0.01; day effect and water × day interaction within the injection treatment, p < 0.05; no water effect).
[4] Day after vitamin administration except d 0 is before administration. Water administration was stopped at d 28 postweaning.

Furthermore, this result means that vitamin E injection diminishes the impact of drinking water administration of vitamin E on serum α-tocopherol concentrations similar to the result of serum 25-OHD$_3$ concentrations. Previously, Wilburn et al [22] reported when dietary vitamin E supplementation level increased, the water administration effect decreased even though water and dietary supplementation of vitamin E had an additive effect. It should be noted that i) the CON group had a continuous decrease of serum α-tocopherol concentration which agrees with Wilburn et al [22], and that ii) there was no difference in serum 25-OHD$_3$ concentrations at d 0 (initial) between non-injection and injection groups whereas serum α-tocopherol concentrations were greater in the injection group which had lower BW than non-injection group.

Even though serum α-tocopherol concentrations increased immediately after vitamin E injection peaking at d 1 post-administration, a large drop occurred between d 1 and 2 post-administration and serum values decreased continuously (Table 7). This result agrees with Jang et al [6] who reported that plasma α-tocopherol concentrations of neonatal pigs peaked at d 1 post-administration by IM injection, and then decreased thereafter.

Regarding the temporal changes of serum α-tocopherol concentrations within the non-injection groups, the water-only group decreased continuously from d 1 to 14 post-administration with a large reduction at d 28 post-administration when vitamin supply in drinking water discontinued whereas the CON group had a continuous decrease from weaning (Table 8). Additionally, serum α-tocopherol concentrations became less than the initial values from d 3 post-administration in the non-injection group and from d 7 post-administration in the injection group regardless of the water treatments. Jang et al [5] reported that plasma α-tocopherol concentrations of pigs at d 14 postweaning were lower than those at weaning even though vitamin E was supplemented in drinking water from weaning which agrees with the result of the current study.

IMPLICATIONS

Vitamin D_3 status of weaning pigs can be enhanced by administration of vitamin D_3 either by IM injection or in drinking water. An additional increase of serum 25-OHD$_3$ concentrations was observed by drinking water administration when pigs were injected with vitamin D_3. The increment of serum values by water administration for the injected pigs was lower than the non-injected pigs, which means that IM injection dominates the effect of enhancing vitamin D_3 status against water administration. Vitamin E status (serum α-tocopherol concentrations) increased dramatically by IM injection of vitamin E immediately after administration but was enhanced slightly by administration of vitamin E in drinking water. Even though vitamin injection could increase vitamin status immediately after administration and be more efficient to enhance vitamin status than water administration in a short term period (acutely), a continuous supply via drinking water is needed to maintain a high vitamin status (chronically). Further studies are needed, however, to determine if vitamin D and E status could be altered by administration strategies different than used herein such as multiple injections, continuous supplementation of vitamins in drinking water and variable supplementation rates in the diets.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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