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Enhancement of selectivity, 25-hydroxyvitamin D3 level, alkaline phosphatase activity and reproductive performance in gilts and primiparous sows using 14-epimer of 25-hydroxyvitamin D3

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ABSTRACT

Selecting breed-worthy gilts as sow replacements is essential for continuity of pig production cycle. Though vitamin D3 (VD3) is known to enhance reproductive performance of multiparous sows, there is still a knowledge gap on its impact in developing gilts and primiparous sows. This study was aimed to quantify plasma 25-hydroxyvitamin D3 (25(OH)D3), serum alkaline phosphatase (ALP), and examine the reproductive performance of primiparous sows fed diets supplemented with regular VD3, and its 25(OH)D3 epimers. The study sample comprised 10-week-old replacement gilts (50 % *Landrace* x 50 % *Yorkshire, N* = 180) assigned in a randomized complete block design to three treatments [2,000 IU/kg of VD3 (T1), 25 μg/kg of 14-epi-25(OH)D3, half dose (T2), and 50 μg/kg of 25(OH)D3 (T3)] equilibrated to 2,000 IU/kg in base diets. Selections occurred at 22, 27 and 35 weeks of age, respectively. Plasma 25(OH)D3, serum alkaline phosphatase (ALP), bone structure and reproductive performance were analyzed. Dietary treatments influenced carpus ($P = 0.023$), fore view stance ($P = 0.017$), infantile vulva ($P = 0.014$), inverted ($P = 0.048$), and prominent teat ($P < 0.001$). Post-partum 25(OH)D3 concentration and ALP activity were elevated by day 25 ($P < 0.001$). Treatment diets also influenced total born ($P < 0.001$), born alive ($P = 0.048$), and still born ($P = 0.049$). Two factors affect circulating 25(OH)D3 and ALP activity: physiological changes in sows during lactation, and dietary 25(OH)D3 intake. 14-epi-25(OH)D3 is a potent metabolite for improving maturation of reproductive organs in developing gilts. It also reduces still birth in primiparous sows.

1. Introduction

The need for good growing, structurally sound, breed worthy replacements for sows exiting a breeding herd is the central objective of gilt development. Careful handling, proper management, as well as recurrent evaluation of structural and reproductive traits are some of the major practices during development for improving the quality of a breeding herd (Patterson & Foxcroft, 2019). Studies have shown that reproductive problems and bone related ailments are leading causes of early involuntary culling (Stock et al., 2018; Yatabe et al., 2019). Gilts therefore require sturdy bones to accommodate weight increase over time and ease locomotion (Stalder, 2006). Vulva must be well-developed, and tipped downward to support mating; breast teeth must also be prominent, properly aligned on opposite sides of udder and preferably more than seven pairs (Safranski, 2016; Stalder et al., 2005). Another common practice during gilt development is the use of dietary supplements (Lauridsen et al., 2010). A typical example is cholecalciferol/vitamin D3 (VD3); its role in ALP activity and gilt performance is fundamental to this study.

Dietary VD3 is hydrolyzed by hepatic hydroxylases to 25-hydroxyvitamin D3 (25(OH)D3), a stable metabolite often used as an indicator of VD3 status in blood (Kolp et al., 2017). Renal form of this metabolite exists as calcitriol (1,25(OH)2D3), an active hormone which co-regulates circulating 25(OH)D3, Ca-P homeostasis and various enzymes; a typical example being 'the alkaline phosphatase' (ALP) (Heaney, 2008; Kiran et al., 2014). These group of enzymes are ubiquitously expressed in skeletal, hepatic, and renal tissues (Shaheen et al., 2012). They are also actively involved in bone matrix mineralization (Schoppet

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& Shanahan, 2008). Studies have shown that 25(OH)D3 is a feedback regulator of ALP enzyme (Halline et al., 1994; Noda et al., 2017). Though its downstream metabolite, 1,25(OH)2D3 promotes the enzyme transcription, the activity of ALP can be inhibited in 25(OH)D3 deplete state; this has been implicated in ailments such as rickettsia and osteomalacia (Arnold et al., 2015; Jesudason et al., 2002).

Following its approval as safe for animals by food and drug administration in 2007, more commercial variants of 25(OH)D3 have been synthesized from VD3 and 25(OH)D3 by modification of its side chain Smith (2007). Earlier attempts to modify the side chain of 25(OH)D3 were marred by inactive stereo isoforms than the parent compound (Maynard et al., 1994). However, the use of bioengineered strain of *Saccharomyces cerevisiae* to sequester 25(OH)D3 from VD3 culture has led to successful production of 25(OH)D3 to meet the dietary demand of pigs especially in intensive production facilities (Chung et al., 2014; Smith, 2007). The dynamics in production technology has led to plethora of 25(OH)D3 epimers whose biological functions in replacement gilt and primiparous sows has not been fully understood. Though Dietary doses of 25(OH)D3 required for sufficiency has been reported (Chung et al., 2014; NRC, 2012), there has been inconsistent in literature regarding the potency and biological function of various products. While some scholars reported that 25(OH)D3 is a more potent form (Burild et al., 2016; Flohr et al., 2016; Lauridsen et al., 2010), others maintained that regular VD3 can be used in lieu of 25(OH)D3 without jeopardizing pig performance (Jakobsen et al., 2007). Some of the known sources of disparity include production stage, season, dietary calcium and phosphorus levels, and conformation of epimers (Arnold et al., 2015; Okafor & Homwong, 2024; Sawada et al., 2010; Tang et al., 2020). Therefore, the objectives of this study were to quantify plasma 25(OH)D3, alkaline phosphatase (ALP) and examine bone structure in developing gilts as well as reproductive performance of primiparous sows fed diets supplemented with regular VD3 and different epimeric conformations of 25 (OH)D3.

2. Materials and methods

2.1. Ethics approval

This project was conducted under the approval of the Institutional Animal Care and Use Committee of Kasetsart University, Thailand.

2.2. Experimental design and dietary treatments

In a randomized control block design, two batches of 90 crossbred gilts (50 % *Landrace* x 50 % *Yorkshire*) averaging 10 weeks of age and 29.5 ± 1.7 kg body weight were randomly assigned (in 30′s per pen) to one of three treatments: a control diet (T1) containing 2000 IU/kg of VD3, 25 μg/kg of 14-epi-25(OH)D3 (Bio D®) (T2) or 50 μg/kg of 25(OH) D3 (Hy-D®) (T3). The three commercial products were equilibrated to 2000 IU/kg as fed in the base diet.

During development, gilts were offered dietary treatments *ad libitum* in three phases: replacement gilt diet 1 (phase 1, weeks 10 to 15 of age), replacement gilt diet 2 (phase 2, weeks 16 to 28 of age) and gestation diet (phase 3, weeks 29 to 35 of age). The fourth phase (lactation diet) was introduced 10 days prior to parturition for acclimation and continued for approximately 25 days through lactation period. The nutrient compositions of phases 1 to 4 diets were formulated using FeedLIVE® Version 1.61 (Live Informatics Co., Ltd., Nonthaburi, Thailand) as shown in Table 1.

2.3. Environment and housing

The gestation and lactation barns were equipped with evaporative cooling systems. Developing, gilts were housed in groups (pen dimension = 4.7 × 8.5 m^2). During gestation, sows were housed individually in stalls. From day 110, sows were moved to a farrowing barn. Individual farrowing stalls were equipped with heated crates and a creep area. Sows and piglets had unlimited access to drinking water in their respective stalls by nipple. Additionally, water was supplied regularly in bowls for easy access to younger piglets. Where necessary, sows were assisted by injecting oxytocin during farrowing. Piglets were weighed within 24 h of farrowing. Cross-fostering was carried out within 48 h after farrowing; however, movement of piglets between crates was restricted to sows farrowed within treatment groups in the same barn.

Table 1
Composition of diets and their respective nutrient specifications.

Ingredients,%	Diets (as-fed basis)			
	Phase 1	Phase 2	Phase 3	Phase 4
Broken rice	46.00	35.00	10.00	35.00
Tapioca meal 70 %	—	12.41	30.00	5.00
Rice bran	22.63	10.00	23.29	15.00
Wheat bran	—	11.00	15.00	12.00
Soybean oil	2.93	3.86	1.99	5.70
SBM 45.5 %	23.00	24.00	15.78	22.44
Fish meal 56 %	2.00	—	—	—
L-lysine	0.34	0.30	0.15	0.65
DL-methionine	0.20	0.22	0.08	0.13
L- threonine	0.14	0.15	0.07	0.09
Monodicalcium phosphate	1.01	1.27	1.08	1.41
Calcium carbonate	1.01	1.01	1.79	1.80
Salt	0.23	0.28	0.25	0.27
Premix[1¶]	0.50	0.50	0.50	0.50
Optiphos® 5000	0.01	0.01	0.01	0.01
Nutrients,%				
ME, kcal/kg	3300.00	3230.00	2950.00	3300.00
Crude protein	18.50	17.37	14.00	17.50
Crude fat	6.94	6.22	6.22	8.79
Crude fiber	4.12	4.41	5.97	4.71
Calcium	0.90	0.85	1.15	1.15
Total Phosphorus	0.83	0.75	0.86	0.85
Available Phosphorus	0.45	0.42	0.41	0.45
Sodium	0.32	0.32	0.32	0.32
Total lysine	1.20	1.10	0.80	1.36
Total methionine	0.51	0.47	0.27	0.38
Total methionine + cystine	0.75	0.72	0.48	0.63
Total threonine	0.78	0.75	0.53	0.68
Total tryptophan	0.22	0.22	0.17	0.21

[1] The premix contents (calculated as unit per ton of feed) include: vitamins A (15,000,000 IU), vitamin B12, thiamine, riboflavin, pyridoxine, VD3 (T1: 2000,000 IU), 14-epi-25(OH)D3 (T2: 25 mg), 25(OH)D3 (T3:50 mg), vitamin E (55,000 IU), vitamin K3 (5.00 g), vitamin B12 (0.04 g), thiamine (2.00 g), riboflavin (6.00 g), pyridoxine (7.50 g), pantothenic acid (20.00 g), niacin (8.00 g), folic acid (1.50 g), biotin (2.00 g), choline chloride (300.00 g), iron (200.00 g), chelated organic zinc (180.00 g), copper (180.00 g) and manganese (54.00 g), cobalt (2.00 g), iodine (2.00 g) and Se (0.30 g). Phases 1 to 4: replacement gilt diet 1, replacement gilt diet 2, gestation and lactation diets respectively. ¶Formulated at Swine Science Laboratory, and manufactured at Animal Supplement & Pharmaceutical Co., Ltd. (Pathum Thani, Thailand).

2.4. Gilt selection

As standard protocol during development, three subsequent selections for structural and reproductive traits at 22, 27 and 35 weeks of age were undertaken. Prior to this process, gilts were moved to a selection booth which was a large space (for easy locomotion) equipped with a weighing balance and used for examination of phenotypic traits. Structural conformation, reproductive traits, locomotion, body weight back fat and health status were physically examined at this stage. The first selection focused on leg conformation, curvature of fore and hind leg pasterns, claw length and size, front and rear-view stance, number and conformation of breast teat, body weight and locomotion. At week 27, selection for breast teat conformation, vulva length, and conformation, locomotion and growth rate was carried out. At week 35, all structural and reproductive traits including locomotion, back fat, body length, heart girth, waist circumference and body weight were

examined. With exception of BW, back fat, vulva length, and number of breast teats, each body condition was assigned an ordinal score. A body condition score cart containing drawings and written descriptions of all anatomical landmarks examined was used as a visual guide during selection (Fig. 1). Gilts were rated according to deviations from normal anatomical conformation. With respect to locomotion, three scores were assigned according to ease of locomotion. Gilts with severe leg problems, under-developed mammary glands, infantile or tipped-up vulva, locomotion problems, sickness, slow growth or perceived as unfit for reproduction were either culled or excluded from entering breeding herd. The term "removal" was used collectively to describe voluntary culls (due to sickness, lameness, locomotion problem) and deaths.

2.5. Records and measures

2.5.1. Environmental records

Barn temperature (°C), humidity (%), and sunlight (lux) were recorded three times per day (9:00 am, 2:00 and 5:00 pm) using an environmental meter (Extech® EN300, Taiwan). These records were used to estimate barn average daily temperature, humidity, and sunlight. .

2.5.2. Performance records

All performance records and inventory in the production facility were digitized in PigLIVE® Version 4.0 (Live Informatics Co., Ltd., Nonthaburi, Thailand). A live record of the insemination date was documented; however, sows were confirmed in-pig 30 days after by a standing reflex during boar exposure. Gestation and lactation length (days) were days from insemination to farrowing and farrow to wean, respectively. Records collected at farrow were farrowing time (hours), which included the time from farrow of first piglet to completion, oxytocin use (ml), and percent oxytocin use. Post-farrowing reproductive performance indices recorded onsite were total born (TB), born alive (BA), still born, mummified, piglets and litter body weight (BW, kg), pre-weaning mortality, weaned piglets, and lactation feed intake (LFI, kg/day). Sow body condition, mainly BW and backfat (BF, mm) before farrowing, and at weaning, as well as percent BW and BF loss during lactation were also measured. Fecal score was recorded daily by observing piglets' fecal droplets in each pen. Stools were assigned 0, 1, 2 indicating lumpy (no diarrhea), pasty, or liquid (diarrhea) stool, respectively. Daily feed intake (kg), number of selected, culled, dead gilts, as well as post-farrowing performance index were also recorded. Average daily weight gain (kg), change in body weight (BW), feed conversion ratio (FCR), 25(OH)D3 concentration (ng/ml), ALP activity (U/L) and treatment Ca level were all analyzed.

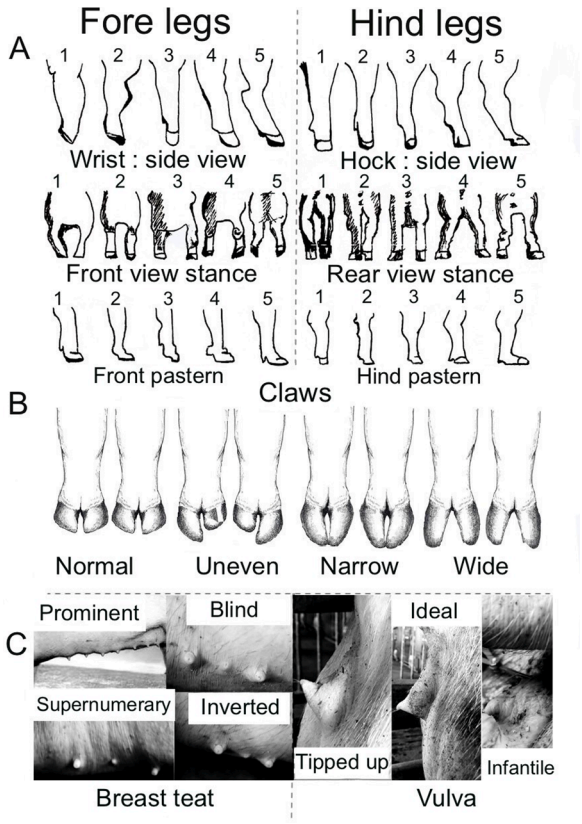

Fig. 1. Visual anatomical guide for evaluating structural and reproductive traits in gilts. The diagram shows a scoring system used for examination of (A) fore and hind legs (B) claws and (C) udder and vulva. The carpus, fore-view stance and fore pastern were examined in fore leg while hock angle, rear view stance and hind pastern were evaluated in hind leg. Normal teats are straight, evenly spaced and prominent. Carpus and hock: extremely bucked (1), bucked (2), normal (3), sickled (4), extremely sickled (5); Fore and rear-view stance: o-shaped (1), standing inward (2), normal (3), standing outward (4), x-shaped (5); fore and rear pasterns: extremely high (1), high (2), normal (3), weak (4), extremely weak (5). NB: One or a combination of the above traits can be found in one udder [Adapted from (CCSI, 2001)].

2.6. Feed analysis

As a quality control measure, five-hundred grams of feed from each batch offered to gilts and sows were sampled for proximate analysis. Dry matter (method 930.15), crude protein (method 2001.11), ether extract (method 2003.05), crude ash (method 942.05), crude fiber (method 978.10), calcium (method 927.02), and phosphorus (method 965.17) were analyzed according to the AOAC protocol (AOAC, 2019). Gross energy was determined using a bombs calorimeter (method ISO 9831). The outcome of the analysis has been presented in Table 2. Quality control analysis for 25(OH)D3 level in Phases 1 to 4 diets was carried out at BIOVET® laboratory (Peshtera, Bulgaria).

2.7. Blood sample collection

This process was carried out via jugular venipuncture at five different stages with unequal interval: **Entry** (day 1 in replacement barn), 35 weeks of age (**W35**), five days post-farrowing (**AF5**), 25-days post-farrowing (**AF25**), and 6 days post weaning (**AW6**). Piglets' blood samples were collected seven days post weaning (**PG7**). Blood samples to be processed into serum were collected in coagulant tubes and plasma in EDTA-infused anticoagulant tubes. All samples were centrifuged at 2500 x g for 15 min at 4°C; the resulting serum and plasma were carefully transferred to 1.5 ml microtubes, labeled, and stored in −20°C for further analysis.

2.8. Analysis of 25(OH)D3 concentration

2.8.1. Sample preparation

Plasma and acetonitrile were added dropwise into microtubes at a ratio of 1:2 (v/v plasma/acetonitrile) and mixed thoroughly by a vortex. The preparation was centrifuged thrice at 5000 x g for 10 min. The supernatant was collected in clean microtubes, passed through a nylon filter membrane (0.22 μm, Whatman) onto an amber glass vial and transferred to an autosampler for chromatographic analysis.

2.8.2. Chromatography

Reverse Phase Symmetry C18 column, (5 μM 4.6 × 250 mm, Waters, USA) was used. The mobile phase contained 100 % acetonitrile, delivered at a flow rate of 1.2 ml/minute. Sample injection volumes were set at 50 μl. Injector and column temperatures were set at 25 and 40°C, respectively. Chromatographic separation occurred at a detection wavelength of 264 nm using a photodiode array (PDA) detector (Waters, San Ramon, CA).

Table 2
Proximate analysis showing the respective treatment composition of various nutrients in diets (Phase 1 to Phase 4).

Feed	Replacement diet 1 (Phase 1)			Replacement diet 2 (Phase 2)			Gestation diet (Phase 3)			Lactation diet (Phase 4)		
Treatments	T1[‡]	T2[†]	T3[§]	T1[‡]	T2[†]	T3[§]	T1[‡]	T2[†]	T3[§]	T1[‡]	T2[†]	T3[§]
Gross energy, kcal/kg	4695.35	4732.22	4749.98	4643.18	4748.12	4672.00	4486.41	4615.29	4636.20	4307.20	4222.12	4208.42
Crude protein,%	20.62	20.88	20.92	18.21	19.20	19.02	15.30	15.37	15.09	19.49	18.79	18.93
Ether extract,%	9.00	8.68	9.15	7.29	7.51	6.82	7.78	8.26	7.85	12.54	11.78	12.23
Crude fiber,%	2.41	2.23	2.18	2.67	3.55	3.26	4.32	4.67	4.31	3.91	3.46	3.98
Crude ash,%	6.69	6.51	6.47	5.36	5.99	5.82	7.46	7.07	7.12	7.50	7.19	7.11
Calcium[1],%	0.97	0.93	0.91	0.91	0.89	0.81	1.29	1.22	1.18	1.39	1.35	1.27
Phosphorus,%	0.85	0.84	0.86	0.57	0.73	0.67	0.81	0.81	0.80	0.96	0.89	0.87

[1] Calcium were analyzed by using Atomic Absorption Spectrophotometry (AA-7000, Shimadzu).
[‡] T1: Basal diet with Vitamin D3 2000 IU plus Optiphos® 0.1 g per kg diet,.
[†] T2: Basal diet with 25 μg 14-epi-25(OH)D3 of Bio D® plus Optiphos® 0.1 g per kg diet and.
[§] T3: Basal diet with 50 μg 25(OH)D_3 of Hy-D® per plus Optiphos® 0.1 g per kg diet.

2.8.3. Calibration curve

Standards of VD3 and 25(OH)D3 (Ehrenstorfer GmbH, Augsburg, Germany) were used to generate calibration curves. Using the mobile phase as diluent, 128, 64, 32, 16, 8 and 4 ng/mL of VD3 and 25(OH)D3 were prepared and used as calibrators.

2.9. Analysis of ALP activity

The *in vitro* test for the quantification of serum ALP activity was carried out using an ALP kit (Mindray, China). The reagents consisted of the following: R1, magnesium acetate-zinc sulfate in a solution of 2-amino-2-methyl-1-propanol buffer (2.5, 1.2 and 435 mmol/L, respectively) and R2, *p*-nitrophenyl phosphate (60 mmol/L). Multi-sera calibrators and controls were used for quality control assessment (reference range: 80.6 - 98.6 U/L). Distilled water was used as blank. Reaction volumes for samples and reagents (sample: R2:R1) were set to 1:12.5:50. Absorbance readings were obtained using a chemistry analyzer (Mindray BS-120, Shenzhen, China) at a reaction temperature of 37°C and wavelengths of 405 – 546 nm. Detection range for linearity was between 5 and 800 U/L. The equation for the reaction was as follows: 4 − Nitrophenylphosphate + H_2O + ALP + $Mg^+ \rightarrow$ 4 − Nitrophenol + P_i. ALP catalyzes the hydrolysis of 4- nitrophenyl phosphate, producing 4- nitrophenol and inorganic phosphate, the alkaline buffer also acts as a phosphate-group acceptor. The activity of ALP is directly proportional to the rate of formation of 4-nitrophenol in the sample.

2.10. Analysis of bone density

2.10.1. Preparation

Forelimbs of culled gilts were sampled. The limbs were undressed and the resulting bone sun-dried. Two metacarpal bones were excised, labeled and sent for computer thermographic scan (CT-scan) at the Faculty of Veterinary Medicine, Kasetsart University. The remaining two were stored in −20 °C for bone Ca analysis.

2.11. Analysis of bone calcium concentration

2.11.1. Preparation of samples and standards

Bone Ca was prepared by dry ash method using metacarpal bones from fore limbs of culled gilts. Two metacarpal bones were defatted (by removing the entire marrow), grounded to fine particles, packed in polybags, and labeled appropriately. Each bag was spiked with petroleum ether at a dilution factor of 10 (dry weight:volume) for 16 h. From each bag, 2 g of bone was weighed into a crucible and heated to ash at 600 °C for approximately 2 h (until fumes disappeared). Calibration standards were prepared by diluting a stock solution of 1 mg/ml calcium nitrate Ca (NO_3) (Certipur, Merck, Germany) to the following concentration: 0, 0.001, 0.002, 0.003, 0.004 and 0.005 mg/ml. The releasing agent solution, 0.1 % lanthanum oxide La_2O_3 (analytical grade, Merck, Singapore), was freshly prepared daily and used as diluent for both samples and standards.

2.11.2. Extraction and estimation of calcium level in bone

Bone ash was washed into a beaker containing dH_2O, 65 % HNO_3 and 37 % HCl at the ration of 2.5:1:1, respectively. Both samples and blanks (dH_2O) were condensed (by evaporation on a hot plate) to quarter of the original volume. On cooling, all solutions were filtered into a clean container containing filter paper (Whatman) and diluted 15x using 0.1 % La_2O_3. Absorbances were read at a wavelength of 422.7 nm using an atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) with flame automation.

2.12. Statistical analysis

General linear mixed-effects model for analysis of ALP and 25(OH) D3 was as follows:

$Y_{ij} = \mu + Z + TR_i + PR_j + TR_i{:}PR_j + e_{ij}$; where Y_{ij} = vector of response for any group of randomly selected gilts in i^{th} treatment at j^{th} period. Where μ = grand mean, Z = random effect, TR_j = treatment effect, PR_j = period effects, $TR_i{:}PR_j$ = interaction between treatment and period effects, and e_{ij}, the residual error. For analysis of bone Ca concentration, treatment was a fixed effect and selection batch a random effect in the model. To reduce variance in standard error of mean, bootstrapping technique was implemented (Roos et al., 2016).

To analyze ADFI, the selection batch and number of gilts were random terms in the model: $Y_{ij} = \mu + Z_b + TR_{ij} + e_{ij}$; where Y_{ij} = vector of response for i^{th} gilt in j^{th} treatment, μ = grand mean, Z_b = unique random intercept for the selection batch. The following model was used for analysis of ADG: $Y_{ij} = \mu + Z_b + Z_{days} + TR_{ij} + e_{ij}$; the model was adjusted for number of days fed (represented as Z_{days}). Total born, born alive and still birth used the model form: $Y_{ij} = \mu + Z_{pbw} + TR_j + e_i$; where the term Z_{pbw} represents the random effects due to piglet birth weight.

Treatments ALP activity and 25(OH)D3 concentration by period, FCR, body weight, back fat, age, vulva length, number of breast teats, oxytocin use, delayed farrowing, pre-weaning mortality, change in body weight and back fat, as well as lactation feed intake were all analyzed using general linear regression model of the form: $Y_{ijk} = \mu + Batch_j + TR_i + e_{ijk}$; where μ = grand mean, TR_i and $Batch_j$ = treatment and batch effect, e_{ijk} = residual effect.

Reproductive traits and selection decisions were analyzed using binomial distribution with "probit" link according to the model: $\Phi^{-1}[\hat{p}ij] = \mu + Batch_j + Tr_i$. Where $\Phi^{-1}[\hat{p}ij]$ is a latent binomial response variable (transformed by probit link function), $Batch_j$ and Tr_i = batch and treatment effects respectively. Death incident was analyzed using quasibinomial logistic regression (Homwong et al., 2016). Structural traits and piglets' fecal scores were analyzed using multinomial logistic regression (Agresti, 2018).

Trend in environmental temperature, humidity and light was analyzed and fitted using locally weighted regression "loess" as follows: $\hat{g}(x) = \sum_{i=1}^{n} l_i(x) y_i$; where g ϒ(x), the fitted value, is a linear

combination of the response variables (temperature, humidity or light) measured at any given time. The term, $l_i(x)$ is dependent on the sum of x_k for k = 1- n^{th} term, weight of observation and Euclidean distance between fraction of points close to fitted value g ̑(x) (Cleveland & Loader, 1996).

Survival probability was estimated using the Kaplan-Meier survival function (Austin, 2017). Culled and dead gilts were the incidents; gilts that survived past the observation time (161 days) were censored.

Assumptions for normality, linearity or heteroskedasticity were tested in linear models. In cases of non-normality, transformation power was estimated using Box-Cox procedure (Long & Teetor, 2019). Model selection was carried out using Akaike Information Criteria (AIC) where lower AIC means better model fit. All statistical analyses were carried out using R version 4.2 (R Core Team, 2022). The Tukey's multiple method was used for mean difference comparisons. Statistical significance was set at $P < 0.05$.

The R packages used included: "lme4" for the linear mixed-effect models (Bates et al., 2015), "boot" for parametric bootstrapping technique (Canty, 2002). The package, "labdsv" was used for generalized linear and multinomial logistic regression (Roberts & Roberts, 2016), model assumptions and transformations (such as boxCox) were analyzed using "MASS" package (Ripley, 2011), "agricolae" was used for multiple comparisons (Gomez & Gomez, 1984), "emmeans" was used for mean comparisons (Russell, 2019), "survival" for survival analysis (Austin, 2017), and "ggplot2" for graphical representation (Hadley, 2016).

3. Results

3.1. Feeding and environment

Changes in body weight, back fat, daily weight gain, daily feed intake and feed conversion ratio were not influenced by dietary treatment ($P > 0.05$) (Table 3). Daily weight gain (ADG) during development was approximately 0.9 kg/day; average body weight also exceeded 140 kg by W35 in both treatments and controls (Fig. 2). There was a decreasing trend in light intensity as well as environmental temperature during development. Humidity on the other hand increased during the same period (Fig. 3).

3.2. Gilt selection

Infantile vulva ($P = 0.014$), inverted breast teats ($P = 0.048$) and prominent breast teats ($P = 0.0005$) were the only examined reproductive traits influenced by dietary treatments. Gilts in T2 had the lowest percentage of infantile vulva (4.15 %) and inverted teats (3.12 %). In addition, they also had the greatest percentage of prominent teat (27.69 %) compared to T3 and controls (Table 3). Carpus conformation differed significantly across treatments ($P = 0.023$), with average ordinal score of gilts in T2 leaning towards normal (an ordinal score of 3, Fig. 1). On the other hand, fore view stance tended to lean towards bucked conformation, but differed significantly across treatments ($P = 0.017$).

Two major decisions from the selection process were: 1) decision to keep (for breeding) and 2) decision to cull. Dead animals were also accounted for. At W27, 90 % of gilts were selected in T2 ($P = 0.038$). No further differences were observed with respect to selected, culled and dead gilts (Table 4).

Table 5 shows the various reasons for removals and percentages affected across treatments. Sickness and death had the greatest influence on removal rate accounting for 76.19 %, 46.81 %, and 49.55 % of total removals in T1, T2, and T3 respectively. Gilts in T2 and T3 had the lowest removal rate per factor examined.

The Kaplan-Meier survival plot showing the probability of gilts being removed from herd either due to culls or deaths was estimated (Fig. 4). Approximately 85, 90 and 87 % in T1, T2 and T3 respectively ($P = 0.48$) were censored beyond the median survival time (161 days). Estimated median survival probability was 50 %.

Table 3
Comparing gilts performance, reproductive and structural traits across treatments.

Parameters	Mean			SEM	*P*-value
	T1	T2	T3		
Age at **Entry**, wks	9.72	9.72	9.73	0.10	[3/]0.997
Age at 3rd selection, wks	33.89	33.95	33.99	0.08	[3/]0.761
BW at **Entry**, kg	29.65	29.75	29.14	0.70	[3/]0.808
BW at age 22, kg	84.81	85.07	83.14	1.39	[3/]0.267
BW at age 34, kg	141.29	143.71	139.98	2.18	[3/]0.470
BF, mm	13.80	14.76	14.11	0.29	[3/]0.080
ADG, kg/day	0.92	0.93	0.90	0.08	[4/]0.288
ADFI, kg/day	1.98	2.04	1.97	0.11	[4/]0.725
FCR	2.48	2.58	2.53	0.22	[3/]0.947
Reproductive traits					
Vulva length, cm	3.88	3.72	3.81	0.08	[3/]0.547
Normal vulva,%	85.77	86.51	74.66	5.24	[1/]0.216
Infantile vulva,%	10.89[ab]	4.15[a]	23.07[b]	4.41	[1/]0.010*
Tipped vulva,%	1.86	8.11	1.61	2.45	[1/]0.153
Teat number,%	15.13	15.02	15.20	0.12	[3/]0.374
Normal,%	94.00	100.00	94.00	2.16	[1/]0.065
Evenly spaced teats,%	57.74	69.70	57.38	6.76	[1/]0.324
Straight teats,%	79.34	86.13	88.02	5.03	[1/]0.459
Inverted teats,%	11.30[a]	3.12[b]	16.50[a]	3.96	[1/]0.043*
Blind teats,%	7.99	5.05	14.01	3.83	[1/]0.246
Prominent teat,%	24.47[a]	27.69[a]	3.25[b]	4.83	[1/]<0.001***
Supernumerary teats,%	10.00	10.35	5.55	3.79	[1/]0.591
Structural traits, score[2/]					
Carpus	2.86[ab]	3.02[a]	2.69[b]	0.10	[2/]0.023*
Fore pastern	2.89	3.10	3.00	0.11	[2/]0.256
Fore view stance	2.00[a]	2.16[b]	2.03[ab]	0.05	[2/]0.017*
Fore leg claw	2.20	2.39	2.48	0.17	[2/]0.691
Hock	3.29	3.33	3.26	0.11	[2/]0.737
Hind pastern	2.32	2.36	2.53	0.11	[2/]0.269
Hind view stance	2.13	1.94	2.09	0.09	[2/]0.121
Hind leg claw	1.87	2.00	1.89	0.08	[2/]0.806
Locomotion	1.41	1.44	1.53	0.11	[2/]0.727

[ab] Different superscripts within the same row indicate statistical significance ($P < 0.05$). SEM standard error of mean; ADG average daily weight gain, ADFI average daily feed intake; FCR feed conversion ratio; BW body weight; BF back fat. ♈Values estimated at pen level as gilts were group-fed.
[1/] *P*-value obtained from generalized linear model using probit link function.
[2/] *P*-value obtained from multinomial logistic regression model.
[3/] *P*-value obtained from general linear regression model.
[4/] *P*-value obtained from linear mixed-effects regression model.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.3. 25(OH)D3 concentration

The chromatographic separation of 25(OH)D3 using 100 % acetonitrile as mobile phase had a retention time of 4.70 mins (Fig. 5). The standard calibration curve had a regression coefficient of 0.79 and 0.99 coefficient of determination. At AF25, the concentration of 25(OH)D3 increased 300 % above the initial value at W35 for all treatments ($P < 0.001$). At treatment level (considering period as random variable in model), dietary 25(OH)D3 improved serum 25(OH)D3 levels better than control, $P = 0.005$ (Table 6). The interaction plot between treatment and period (Fig. 6) shows a concentration of 28.60, 34.61, 55.82 ng/ml; 27.76, 32.69, 53.06 ng/ml; 116.69, 189.14, 219.18 ng/ml; and 127.04, 131.32, 158.05 ng/ml in T1, T2, and T3 at W35, AF5, AF25, and AW6 respectively. Only a slight change was observed in treatment concentration between W35 and AF5; however, at AF25 there was an increase in plasma concentration across all treatments which gradually declined at AW6.

3.4. ALP activity, Ca concentration and bone tomography

In Table 6, there were no significant differences in ALP activity at Entry and W35. However, post-partum levels differed significantly.

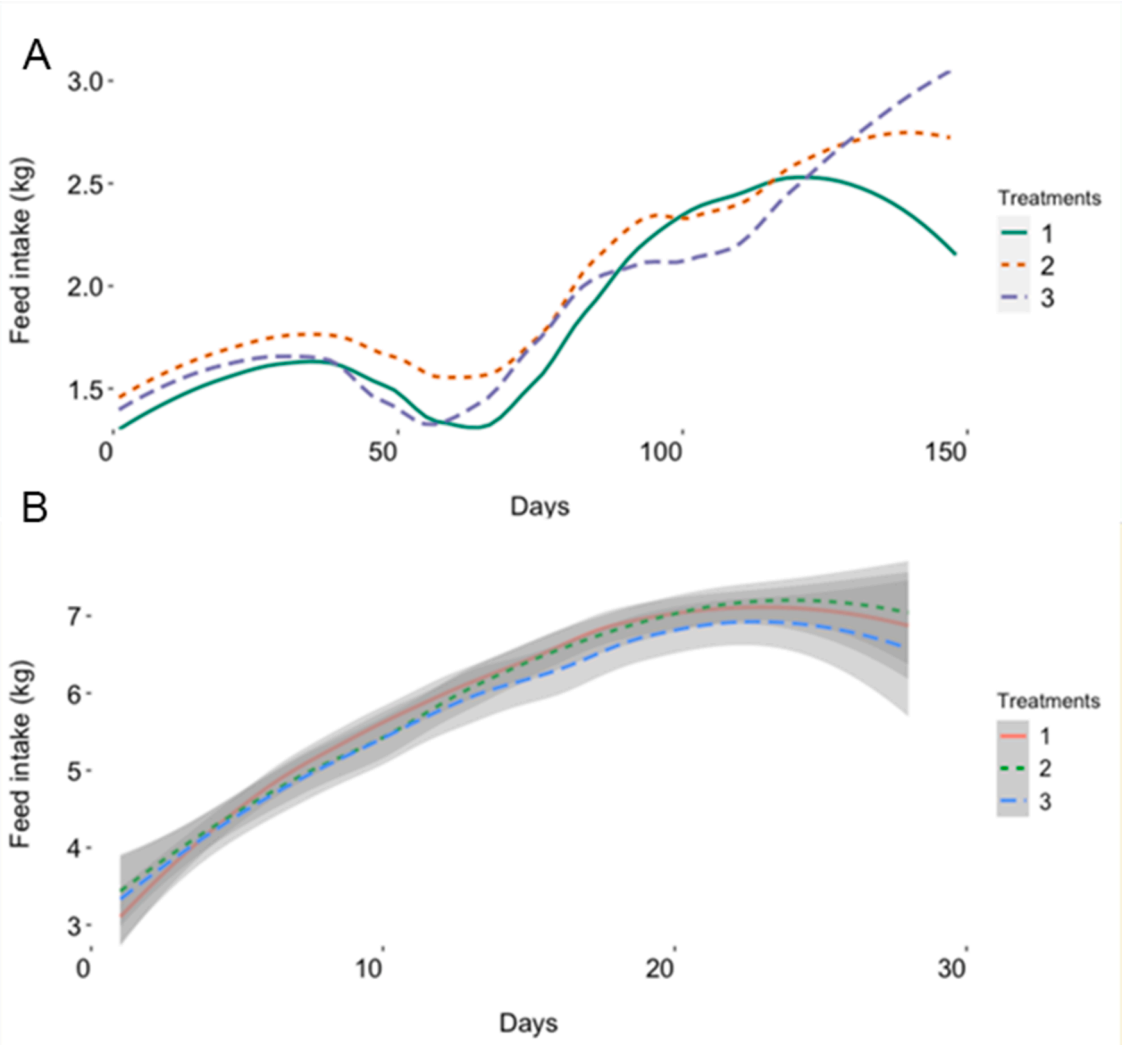

Fig. 2. Comparing average daily feed intake in replacement gilts and primiparous sows by treatment. A Daily feed intake from Entry to W35. B Daily feed intake during lactation (average lactation length was 25 days). Feed intake increased to approximately 2.5 kg/day between the first 100 days in replacement barn. During lactation, feed intake also increased simultaneously with lactation length.

There was a positive increase between AF5 and AF25 ($P < 0.001$). Treatment levels remained statistically significant at AW6 ($P < 0.001$), despite the decline in ALP activity at this period. The interaction plot showed an activity of 178.90, 153.48, 165.47 U/L; 89.82, 77.79, 143.66 U/L, 53.55, 48.62, 99.65 U/L, 130.95, 76.93, 216.61 U/L; and 77.70, 64.67, 128.83 U/L in T1, T2, and T3 at Entry, W35, AF5, AF25, and AW6 respectively (Fig. 7). Piglets had the highest level of ALP activity uninfluenced by any dietary treatment.

There was no significant difference in bone Ca concentration across treatments. Fig. 8 showed cross sectional (A) and three-dimensional (B) tomography of the metacarpal bones. Neither lesions nor physical differences were observed in the periosteum; the width of compact tissues layers were also indifferent across treatments.

3.5. Reproductive performance

Total born, born alive, still born and percent change in body weight were the only measured reproductive parameters influenced by dietary treatments (Table 7). While the control had an average of 15.51 total born ($P < 0.001$) and consequently 13.67 pigs born alive ($P = 0.048$), still born was 1.55 piglets ($P = 0.049$), a 37 % and 105 % above to the values in T2 and T3 respectively. Percent change in body weight was also lower (10.85 %) in controls compared to treatments ($P = 0.011$).

4. Discussion

To the best knowledge of the authors, this was the first study in Thailand investigating the effects of supplementing gilt diets with regular VD3 and two epimers of 25(OH)D3 on plasma 25(OH)D3 concentration, ALP activity, and bone status during development. The effects of these dietary treatments on reproductive performance of primiparous sows were studied as well.

4.1. Feeding and environment

Gilts performance can be deemed efficient with ADG of 0.9 kg/day, ADFI of approximately 2.0 kg/day and FCR of 2.5. The current ADG accedes to the 0.8 kg/day reported for good performing gilts (Johnston et al., 2013). The daily feed intake can also be considered appropriate for gilts of similar physiological stage across various studies (Liu et al., 2020; Stalder et al., 2000; Zebua et al., 2017). Considering the 2.5 ratio estimated herein, though lower than FCR reported by Liu and colleagues, there is a consensus with the range reported in another study (Stalder et al., 2000). It has also been reported that ADFI and ADG are influenced by feeding mode (Liu et al., 2020). In the current study, though gilts had unrestricted access to feed during development, the reduction in dietary energy at each phase was aimed at maintaining a

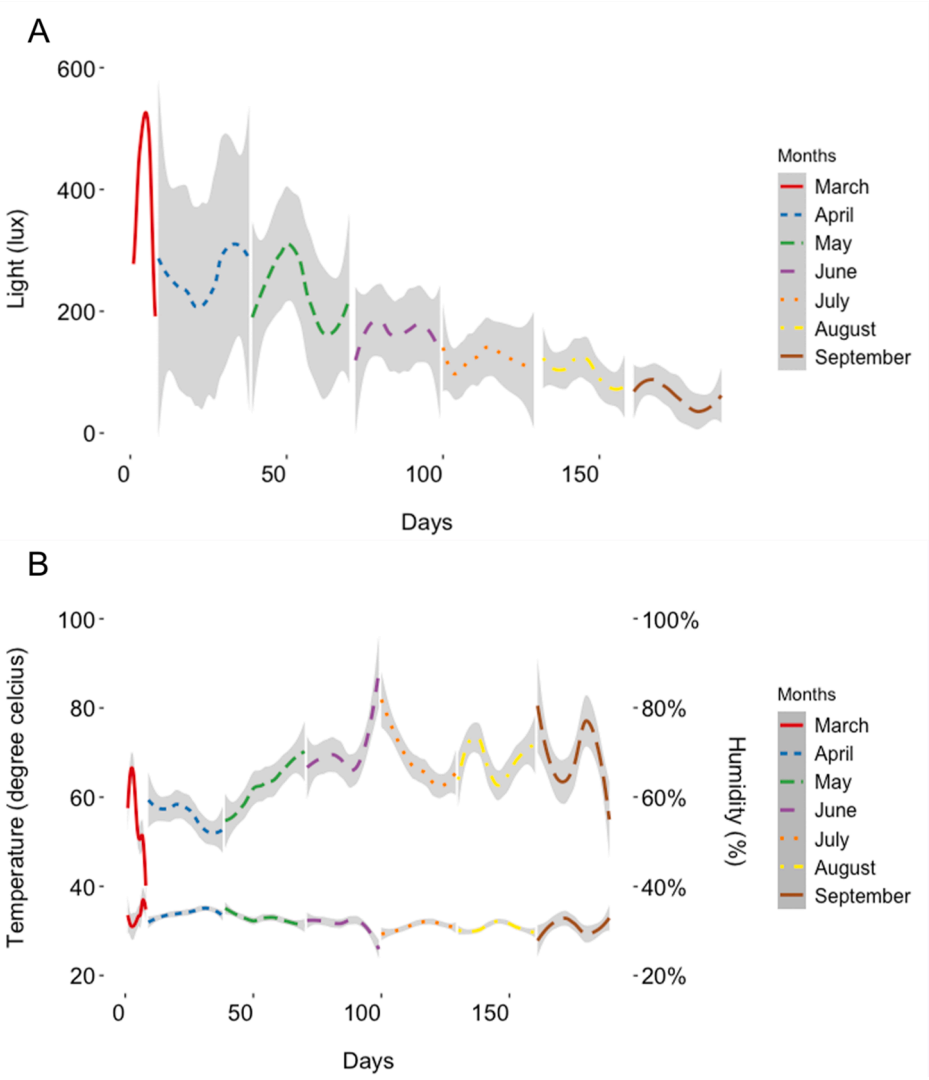

Fig. 3. Trend in environmental temperature, light and humidity during gilt development between the months of March and September. A: Sunlight radiation (lux) B: Temperature (below, °C) and humidity (above,%). Light radiation was at its peak in March but showed a decreasing trend as the months advanced. Temperature showed similar trend as light, with highest record in March but varied less through September. Humidity on the other hand, increased exponentially, from approximately (55 %) in March to a peak above (80 %) in June.

Table 4
Outcome of selection process expressed as percentage of gilts selected, culled and dead across treatments.

Parameters	Mean			SEM	[1/]*P*-value
	T1	T2	T3		
Selected gilts,%					
1st selection	81.68	90.18	86.44	4.42	0.398
2nd selection	73.33[a]	90.16[b]	86.44[ab]	4.66	0.038*
3rd selection	65.04	72.15	72.97	5.90	0.586
Culled gilts,%					
1st selection	4.59	5.01	9.64	3.12	0.481
2nd selection	12.92	5.05	9.70	3.67	0.303
3rd selection	21.34	22.87	23.19	5.41	0.967
Dead gilts,%					
1st selection	12.59	4.8	3.25	3.12	0.108
2nd selection	0.00	0.00	0.00	0.00	NE
3rd selection	0.00	0.00	0.00	0.00	NE

[ab] Means within the same row with different superscripts differ ($P < 0.05$). SEM standard error of mean. NE not estimable.
[1/] *P*-value obtained from generalized linear model using probit link function.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 5
Removal causes and percentage of gilts affected across treatments.

Parameters	Mean			SEM	[1/]*P*-value
	T1	T2	T3		
Causes of removal,%					
Death	12.59	4.80	3.25	3.12	0.108
Lame	0.00	0.00	0.00	0.00	NE
Splay feet	0.00	3.13	5.07	1.70	0.113
Reproductive problem	3.23	1.48	0.00	1.28	0.245
Sickness	13.33	8.24	10.11	3.95	0.657
Underweight	2.65	5.80	4.43	2.60	0.672
Miscellaneous	1.53	1.66	1.55	1.61	0.998
Total removal	34.02	27.86	26.96	5.90	0.577

[1/] *P*-value obtained from generalized linear model using probit link function. SEM standard error of mean. NE not estimable.

balance between growth rate and muscle mass accretion. The environment on the other hand played an important role in regulating internal temperature. A study on the impact of environmental condition on gilt performance reported an optimal temperature below 31°C for enhanced performance under tropical condition (Okafor & Homwong, 2023).

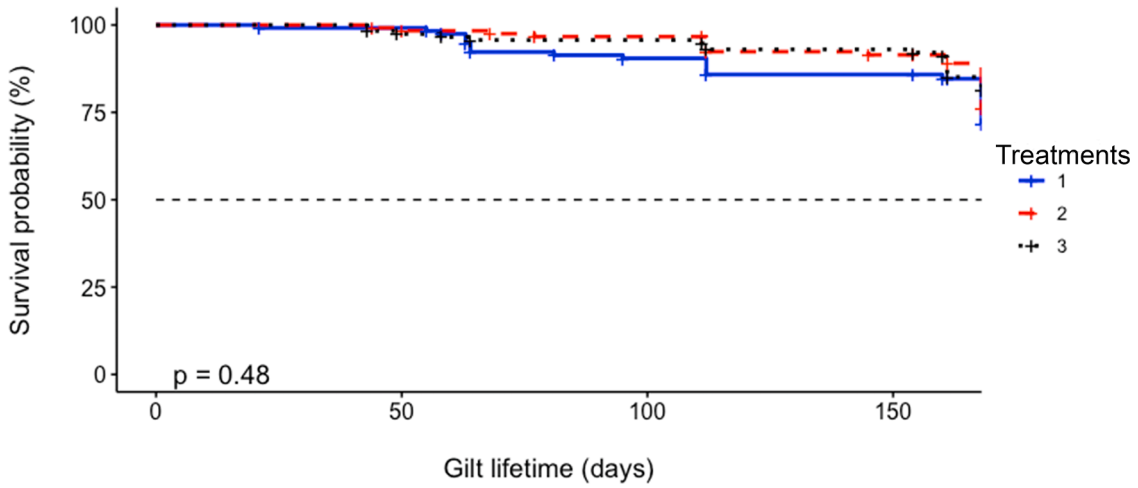

Fig. 4. Kaplan-Meier estimator showing gilt survival probability within the development period. The survival plot was estimated over a period of 161 days which include the total period between entry (at the age of 10 weeks) and third selection (at the age 35 weeks). Median survival time was estimated at 50 %. Removals include number of deaths and/or involuntary culls within this period. Most gilts exceeded the median survival time; gilts in T1 had mean survival probability of 85 %, while those in T2 and T3 had approximately 90 and 87 % respectively. Survival endpoint was not reached as the trial was censored beyond 161. .

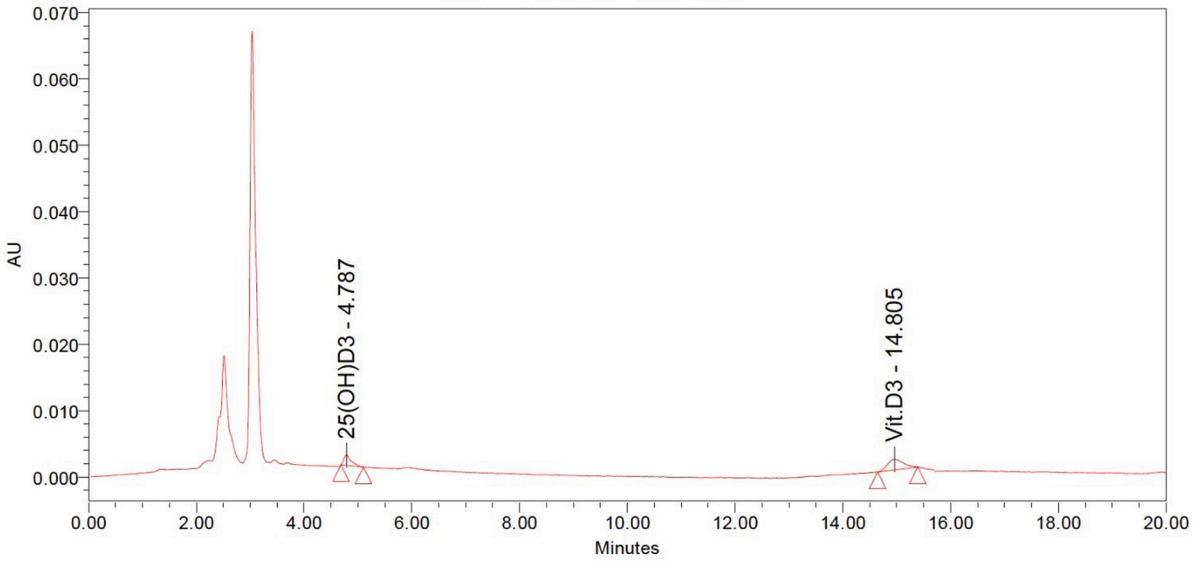

Fig. 5. Chromatogram showing the retention time and separation peaks for the extraction of VD3 and 25(OH)D3. A cocktail was prepared from 50 ng/ml dry mass of VD3 and 25(OH)D3 standard; separation was carried out using 100 % acetonitrile as mobile phase. 25(OH)D3 and VD3 had retention times of 4.79 and 14.81 respectively.

Though the decrease in light intensity due to seasonal shifts helped to stabilize environmental temperature, the use of evaporative cooling system maintainedinternal temperature at gestation and lactation barns between 25-28°C for most part of the study, reducing the possibility of heat stress.

4.2. Gilt selection

Infantile vulva, inverted teat, prominent teat, carpus and fore view stance differed significantly across treatments. An ordinal score of 3.00 was considered ideal for carpus and fore view stance. Traits for carpus conformation was widely distributed across the 5-point scale resulting to the predominant normal observed herein. However, with fore view stance, the uneven distribution of traits resulted to an average score leaning towards the trait, standing inward. Similar finding was observed in another study (Nikkilä et al., 2013). This shortfall often results to misinterpretation of categorical scores during evaluation and should be carefully avoided. Notwithstanding, carpus and fore view stance were in good shape and posed no observable threat to gilt selection. Another important observation is the hind pastern which, indicated that the dominant trait leaned towards “high”. Carpus and fore view stance were reported to have heritability estimates of 0.16 and 0.12 respectively, while pastern has a heritability of 0.42 (Bennett & Bunter, 2003). According to Nikkila and co-researchers, heritable genetic traits such as carpus and fore view stance with low heritability are not only affected by genetics but a composition of various other factors, including nutrition (Nikkilä et al., 2013). Studies have revealed that buckled, high, or weak pasterns are risk factors for hampered locomotion (Aasmundstad et al., 2014; Jørgensen, 2000). According to Jorensen, the inability of flexor tendons to stretch concurrently with the bones during growth has been identified as the leading cause of high, upright or buckled pastern. The severity can sometimes become subtle as animals age (Jørgensen, 2000). Weighing the opportunity cost, these gilts could be of more economic value when retained within the herd, hence no gilts were culled in this

Table 6
Period-dependent variability in 25(OH)D3 concentration, alkaline phosphatase activity and bone calcium concentration compared across treatments.

Parameters	Mean			SEM	*P*-value
	T1	T2	T3		
25(OH)D3 **W35**, ng/ml	30.70	34.04	54.85	10.86	[3]0.253
25(OH)D3, **AF5**, ng/ml	30.70	32.69	52.64	11.14	[3]0.285
25(OH)D3, **AF25**, ng/ml	126.69[a]	164.69[a]	226.57[b]	18.09	[3]0.001**
25(OH)D3, **AW6**, ng/ml	128.02	130.06	156.87	15.03	[3]0.305
25(OH)D3, ng/ml[‡]	84.80[a]	106.50[ab]	131.54[b]	8.86	[4]0.005***
ALP **Entry**, U/L	178.90	153.48	165.47	24.13	[3]0.756
ALP **W35**, U/L	89.82	77.79	143.66	24.29	[3]0.155
ALP **AF5**, U/L	51.10[a]	69.89[ab]	77.79[b]	5.85	[3]< 0.001***
ALP **AF25**, U/L	115.64[a]	136.17[ab]	164.21[b]	11.32	[3]< 0.001***
ALP **AW6**, U/L	84.00[a]	97.24[b]	85.31[a]	10.07	[3]< 0.001***
ALP **PG7**, U/L	192.09	157.66	182.54	29.93	[3]0.487
ALP, U/L[‡]	106.18	84.30	150.84	27.34	[4]0.378
Ca in bone, g/100g[†]	20.52	20.52	21.43	0.49	[4]0.393

[ab] Means within the same row with different superscripts differ ($P < 0.05$). SEM standard error of mean, ALP alkaline phosphatase, Ca calcium, 25(OH)D3 25-hydroxy vitamin D3, AF5: five days post-farrowing, AF25: twenty-five days post-farrowing, AW6: six days post-weaning, PG7: seven days post-weaned piglets
[3]/P-value obtained from general linear regression model.
[4]/P-value obtained from linear mixed-effects regression model.
[†]Bone sample used for analysis.
[‡]Conditional models adjusted for period effects. *$P < 0.05$
** $P < 0.01$
*** $P < 0.001$.

regard.

Dietary treatments had significant influence on infantile vulva conformation. More than 90 % of gilts fed 14-epi-25(OH)D3 had large, tipped down vulva. The result of the current study alludes the fact that analogues of 25(OH)D3 exhibit variable biological functions *in vivo* (Sawada et al., 2012). Though moderately influenced by genetics, its inclusion in the selection goal can be considered crucial owing to the fact that small vulva size is often associated with breeding difficulties, reduced litter size, low number of pigs born alive and mummified piglets (Corredor et al., 2021). The distinct vulva conformations at treatment level shows a considerable difference in potency of the VD3 and 25(OH)D3 products administered.

Only 3.12 % of gilts fed 14-epi-25(OH)D3 had inverted teat. This also shows a difference in potency of 25(OH)D3 used. In a similar finding reported by Long and co-researchers, it was shown that inverted teat is a genetic defect that occurs when the fetal mammary epidermis fails to evert prior to birth. The heritability of this trait in gilts according to their study was 0.3 (Long et al., 2010). Defect in the function of relaxin (a hormone expressed abundantly in developing mammary glands), and parathyroid hormones were identified as the major causes of inverted teat (Chalkias et al., 2017; Sherwood, 2004). Though inverted teats can sometimes be redeemed by palpation between 5 and 6 months, or in some cases during early gestation, the inability to evert at early stage of reproduction could be a potential risk for mastitis (Chalkias et al., 2013).

The finding that 27 % of 14-epi-25(OH)D3 fed gilts and 25 % of controls had prominent teats also indicates that variability exists in the potency of the two 25(OH)D3 forms and the control. Teat traits are an important aspect of most breeding programs and have been extensively studied (Chalkias et al., 2013; Li et al., 2021; Long et al., 2010). Hence, improvement of teat quality can be achieved by inclusion in herd breeding objectives for maternal lines (Long et al., 2010). Chalkias and co-workers analyzed genetic traits that affect the development of prominent teats. In their report, they noted that heritability for prominent teats was relatively high (0.6) and can result to additive effect; hence, its inclusion in breeding programs and can be easily achieved by phenotypic selection (Chalkias et al., 2013).

4.3. 25(OH)D3

Quality control standard for 25(OH)D3 in treatment diets was at the expected level with limit of quantitation between 20 and 60 ng/g at 10 % coefficient of variation for all phases. There was a four-fold increase in plasma 25(OH)D3 concentration during lactation. Overall treatment concentration (in the conditional model) was also statistically significant. Lactation levels reported herein exceeded the values reported for

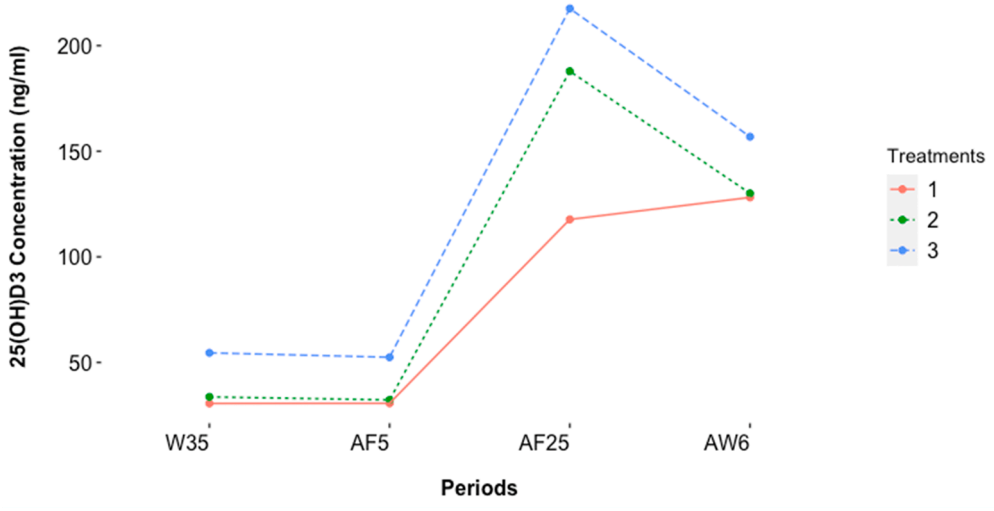

Fig. 6. Effect of treatment and period interaction on 25(OH)D3 concentration in gilts. Entry and W35 represent periods of first and second sample collection (which occurred during development); average age of gilts at these periods were approximately 10 and 35 weeks respectively. AF5 – day 5 post-partum, AF25 – day 25 post-partum, AW6 – day 6 post-weaning. The concentration of 25(OH)D3 was estimated at two production stages: 1) development (for W35) and 2) first parity reproduction (AF5, AF25 and AW6). The interval between sample collection periods is unequal. There was no interaction between treatment groups at all periods. Bioavailable concentration was highest in T3 groups and lowest in T1. Treatments 25(OH)D3 concentration declined slightly from an initial value of 28.60, 34.61 and 55.82 (ng/ml) at W35 to 27.76, 32.69 and 53.06 (ng/ml) in T1, T2 and T3, respectively at AF5. At AF25, these values increased to 116.69, 189.14 and 219.18, declining thereafter to 127.04, 131.32 and 158.05, respectively in T1, T2 and T3 at AW6.

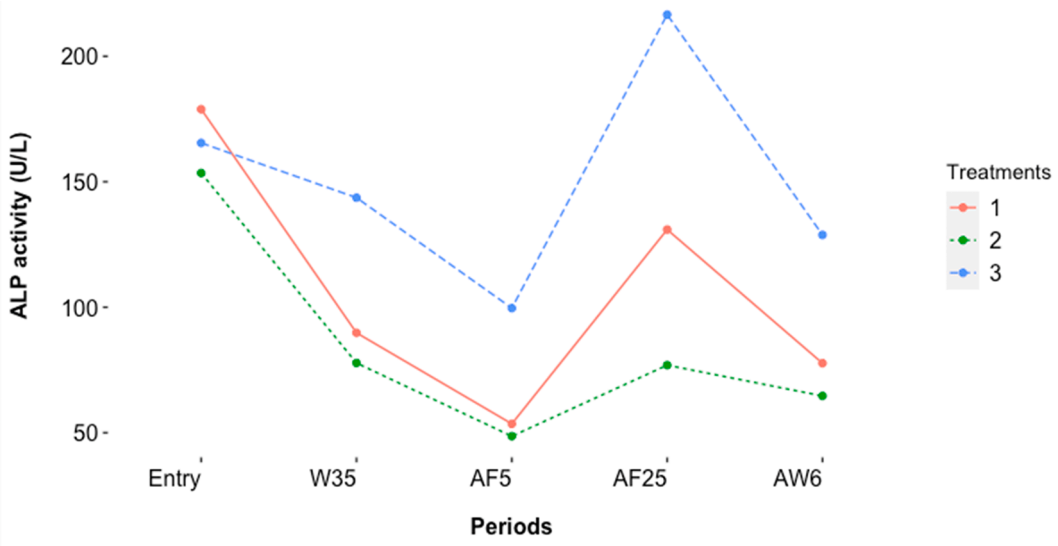

Fig. 7. Interaction between treatment and period on the activity of alkaline phosphatase (ALP). Entry and W35 represent periods of first and second sample collection (which occurred during development); average age of gilts at these periods were approximately 10 and 35 weeks respectively. AF5 – day 5 post-partum, AF25 – day 25 post-partum, AW6 – day 6 post-weaning. Following the blood collection periods, serum ALP was estimated at two production stages: 1) development (for Entry and W35) and 2) first parity reproduction (AF5, AF25 and AW6); however, the interval between each blood collections periods are unequal. Treatment x Period interaction occurred only between T1 and T3 at Entry; no further interaction was observed thereafter. Treatments ALP activity declined from an initial value of 178.90, 153.48 and 165.47 U/L at Entry to 53.55, 48.62 and 99.65 U/L at AF5 in T1, T2 and T3, respectively. All treatment values increased at AF25, reaching a high of 130.95, 76.93 and 216.61 U/L in T1, T2 and T3, respectively. Accordingly, these values declined thereafter at AW6 to 77.70, 64.67 and 128.83 U/L. .

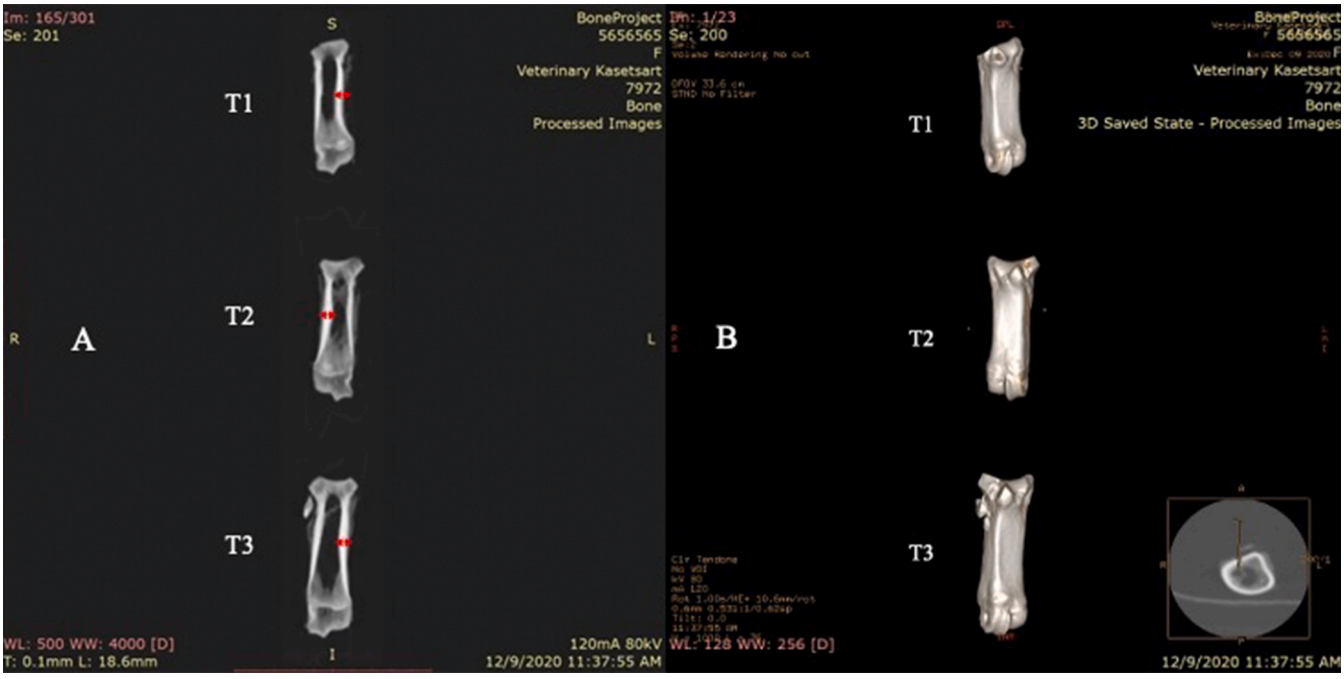

Fig. 8. Comparing the density of calcified layer in metacarpal bones of replacement gilt in treatments. A: cross-sectional view; B: 3-dimensional view. There was no difference in density of calcified layer; no lesion was observed.

both lactating sows and super-dosed piglets (Flohr et al., 2016; Lauridsen et al., 2010). Two factors could be responsible for the observed increase in concentration during lactation: first is dietary feed intake and second, physiological response to serum level of 25(OH)D3, Ca or P. In a previous study, it was shown that in addition to dietary intake, availability of 25(OH)D3 in blood can also be affected by epimeric conformation of 25(OH)D3 in diet (Okafor & Homwong, 2024). The linear increase in plasma 25(OH)D3 concentration with dietary feed intake consents to the finding by Flohr et al. (2016) that bioavailability of 25 (OH)D3 is affected by dietary concentration. The current result also accords previous findings which showed that bioavailability of VD3 and 25(OH)D3 depends on concentration rather than form (Jakobsen et al., 2007; Lauridsen et al., 2010). Wubuli et al. (2020) reported an increase in the activity of 1-alpha hydroxylase during lactation. The activity of this enzyme could be the major contributor to the observed level during lactation. Apart from hydroxylase activity and diet, other factors that influence plasma 25(OH)D3 concentration include plasma melanin, body weight of animal, amount of sunlight as well as Ca concentration

Table 7
Reproductive performance of replacement gilts compared by treatment.

Parameters	Mean			SEM	*P*-value
	T1	T2	T3		
Reproductive performance					
Total born, n	15.51[a]	13.43[b]	13.54[b]	0.54	[4]/< 0.001***
Born alive, n	13.67	12.86	12.93	0.63	[4]/0.048*
Still born, n	1.55[a]	1.13[b]	0.62[b]	0.29	[4]/0.049*
Mummified piglets, n	0.66	0.38	0.33	0.20	[4]/0.446
Piglet weaned, n	12.13	12.25	11.70	0.35	[4]/0.533
Litter total birth weight, kg	15.42	15.57	16.63	1.06	[4]/0.355
Oxytocin,%	51.72	56.25	61.9	10.76	[1]/0.773
Delayed farrowing, days	2.63	3.13	1.90	0.35	[2]/0.078
Change in body weight,%	10.85[b]	15.31[a]	12.23[b]	0.99	[3]/0.011*
Change in back fat,%	8.57	7.48	10.79	1.86	[3]/0.279
Pre weaning mortality,%	4.94	4.63	7.30	1.45	[3]/0.384
Fecal score, score	1.57	1.38	1.30	0.19	[3]/0.604
Lactation feed intake, kg	6.00	6.10	5.83	0.15	[3]/0.494

[ab]Means within the same row with different superscripts differ ($P < 0.05$). BW = body weight, Change in BW% = [(BW before gestation – BW at wean)/ BW before gestation] x 100, SEM standard error of mean.
[1]/P-value obtained from generalized linear model using probit link function.
[2]/P-value obtained from multinomial logistic regression model.
[3]/P-value obtained from general linear regression model.
[4]/P- value obtained from linear mixed-effects regression model.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(Arnold et al., 2015; Del Valle et al., 2011). Stringent efforts were undertaken to eliminate any potential source of bias; for example, only animals of matched age and body weight were included in this study. Also, base diets were formulated as a single batch only varying in VD3 or 25(OH)D3 content thus reducing the chances of overestimating plasma 25(OH)D3 levels due to variable fat content in feed. Dietary Ca was also supplied in the form of calcium carbonate. Notwithstanding, plasma concentration of 25(OH)D3 was above deficiency threshold at all measured periods (Mosekilde, 2005). The downstream effect of 25(OH)D3 concentration on ALP activity, and bone Ca concentration was examined as well.

4.4. ALP, Ca concentration and bone tomography

There was a statistical difference in post-partum ALP activity. The reference range for pigs had been published, and these values vary by age (Cooper et al., 2014). As expected, the activity of ALP was highest in gilts at Entry, and in piglets (PG7). This finding attest to the overt expression of hydroxyapatites known to be peculiar in younger animals during growth (Anderson et al., 2005; Saraç & Saygılı, 2007). However, the values obtained herein exceeds that reported for gilts of matched age in another study (Mayengbam & Tolenkhomba, 2015). Apart from age, other known causes of elevated ALP in the blood include kidney, liver or bone-related ailments (Saraç & Saygılı, 2007). Mutations in the ALP gene has been reported as a leading cause of high ALP activity in blood (Matsuo et al., 2013). This was evidenced when a knockout of the transcription machinery responsible for the synthesis of ALP resulted in hypophosphatasia, and consequently, an increase in ALP activity. Loss of calcemic action of 25(OH)D3 in bone due to reduction or inhibition of 1-alpha hydroxylase activity increases parathyroid hormone level, a potential trigger for an upsurge in ALP activity (Jesudason et al., 2002). It was also reported that elevated ALP is affected by Ca-P and 25(OH)D3 concentration (Wubuli et al., 2020). In this study, ALP activity increased with 25(OH)D3, indicating a possible response to an increase in circulating Ca and P concentration during lactation. At a concentration of 20 ng/100 g of bone ash, bone Ca concentration exceeded that reported for age matched gilts in another study (Varley et al., 2011). Dietary sources are known to have the greatest influence on Ca variability (Reyer et al., 2019; Stein et al., 2011). Sufficiency in physiological levels of 25(OH)D3 in plasma was evidence that Ca levels were adequate to meet the physiological demand for gilts in this study (Papadopoulou et al., 2021).

There were no lesions in the periosteum of the compact tissue. No clinical signs of bone ailments were detected in the CT scans. The width of compact tissue layer was also indifferent across treatments. Having established that the dietary treatments met the nutrient expectations with respect to bioavailability of 25(OH)D3, ALP, Ca, and bone conformation, the effect of dietary treatments on reproductive performance was also examined.

4.5. Reproductive performance

Dietary treatment influenced the number of totals born, still born and change in body weight of primiparous sows. Total born in 25(OH)D3-fed sows (T2 and T3) were 13.4 and 13.5 piglets respectively, similar to that reported by Lauridsen et al. (2010). Though in numerical terms, controls had more total born (15.51), the number of still born (1.55) and mummified piglets (0.66) were also relatively higher. In primiparous sows, still born can be caused by low litter live weight, infantile vulva, difficulty in farrowing as well as sow body dimension (Corredor et al., 2021; Le Cozler et al., 2002). It is well established that uterine vitality and size is essential for mitigating the occurrence of still births and mummies; large uterine provides adequate space for blood flow and fetal development (Père & Etienne, 2000). A study by Le Cozler et al. (2002) revealed that the size of uterus is also associated with sow body dimension, suggesting that sows with larger frames are likely to have more uterine space for fetal development per embryo compared to smaller counterparts. Imperfect maturity of the uterus might therefore be a major cause of the observed number of still born and mummified piglets (Père & Etienne, 2000). According to Le Cozler et al. (2002), short supply of nutrients to embryos distant from the uterine horn during development increases the likelihood of still birth and mummies. This explains why there are relatively higher number of still birth and mummies at the distal end of uterus than towards the horn. Still born can however, be reduced by assisted farrowing (Le Cozler et al., 2002). Lauridsen and co-authors reported that gilts fed greater than 800 IU of VD3, or its 25(OH)D3 equivalent, had lower numbers of still born (Lauridsen et al., 2010). Studies have shown that mummies can be reduced primarily by feeding low energy diets during gestation; this controls excessive accumulation of abdominal fat which is likely to obstruct fetal growth. Hence, mummies are often reduced in litters with low dispersion in live body weight (Le Cozler et al., 2002). Percent loss in body weight is often associated with milking; the net loss in body reserve (usually fat mass) is offset by a positive gain (in growth muscle mass) by piglets; hence, adequate milking will often result to proper development of piglets (Hojgaard et al., 2020). Total number of piglets weaned was numerically higher in 14-epi-25(OH)D3 fed group compared to other treatments, though this value was not statistically significant.

5. Conclusion

This study investigated the effect of feeding various commercial forms of VD3 and 25(OH)D3 on selectivity, ALP activity and reproductive performance in replacement gilts and primiparous sows. The feeding regimen improved gilt body weight from initial average of 29 kg to an average of 140 kg within the 25-week study period. This was an appropriate weight and age for mating. There was no difference attributable to treatment diets on structural traits, however, with reproductive traits, infantile vulva, inverted and prominent teat differed between treatments; this shows variation in potencies of the different commercial VD3 and 25(OH)D3 products used. Gilts were removed mainly as a result of sickness, death, reproductive problems or underweight. Dietary level of 2,000IU of 25(OH)D3 was adequate to maintain blood level above deficiency threshold (30 ng/ml for this study), a concentration

deemed appropriate for normal physiological function. Plasma 25(OH) D3 concentration also increased simultaneously with an increase in feed intake during lactation. The activity of ALP was age dependent with higher levels in younger animals. There was also a simultaneous increase in ALP activity in lactating sows. This could be attributed to the increase in 1-alpha hydroxylase activity during lactation. The present study showed that half dose of 14-epi-25(OH)D3 significantly improved reproductive traits in developing gilts better than regular 25(OH)D3 and VD3 forms. However, epimeric conformation might not be a crucial factor for product choice in primiparous sows.

CRediT authorship contribution statement

Prester C.John Okafor: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nattanit Jimongkolkul:** Visualization, Resources, Methodology, Investigation. **Anchalee Khongpradit:** Visualization, Validation, Methodology, Investigation. **Wunwinee Ahiwichai:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nitipong Homwong:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Recommendations for further studies

An interesting observation was that though scoring systems might differ across studies, strict adherence to judgment standards requires expertise; else, selection results could be misleading. Where possible, one should consider including estimates of the animal breeding value to maximize the benefit of using a particular breed. From both economic and welfare perspective, proper management is paramount for success, not only during gilt development but at all levels of pig production cycle. Future studies will explore the molecular mechanism for 25(OH)D3 action.

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Declaration statement

The authors declare that the manuscript is original, and the results have neither been published nor is under consideration for publication elsewhere. The authors declare no conflict of interest regarding this publication neither is there any financial support that could influence its outcome.

Ethical statement

This project was conducted under the approval of the Institutional Animal Care and Use Committee of Kasetsart University, Thailand.

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