R. Bras. Zootec., 50:e20210098, 2021
https://doi.org/10.37496/rbz5020210098

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Bone traits and gastrointestinal tract parameters of piglets fed cholecalciferol and 1,25-dihydroxycholecalciferol glycoside

Heloíse Trautenmüller[1], Jansller Luiz Genova[1], Adriana Bulcão Bock de Faria[1], Juliana Stocco Martins[1], Sthefany Caroline Marques Viana[1], Leandro Dalcin Castilha[2], Affonso Celso Gonçalves Junior[3], Silvana Martinez Baraldi-Artoni[4], Paulo Levi de Oliveira Carvalho[1]*

[1] Universidade Estadual do Oeste do Paraná, Departamento de Zootecnia, Marechal Cândido Rondon, PR, Brasil.
[2] Universidade Estadual de Maringá, Departamento de Zootecnia, Maringá, PR, Brasil.
[3] Universidade Estadual do Oeste do Paraná, Centro de Ciências Agrárias, Marechal Cândido Rondon, PR, Brasil.
[4] Universidade Estadual Paulista, Departamento de Morfologia e Fisiologia Animal, Jaboticabal, SP, Brasil.

ABSTRACT - The objective of this study was to evaluate the cholecalciferol and 1,25-dihydroxycholecalciferol glycoside supplementation in piglet diet on organ biometry and physicochemical composition, pH of the digestive tract contents, and bone traits. A total of 128 entire male piglets (21 days-old, 6.82±0.38 kg body weight) were distributed in a completely randomized block design with eight replications and four animals per experimental unit. The treatments were composed of a diet 100/0 = 100% of the vitamin D supplemented with cholecalciferol; 50/50 = 50% supplemented with cholecalciferol + 0.25 µg of $1,25(OH)_2D_3$ glycoside; 25/75 = 25% supplemented with cholecalciferol + 0.375 µg of $1,25(OH)_2D_3$ glycoside; and 0/100 = 0.50 µg of $1,25(OH)_2D_3$ glycoside. The results indicated that piglets fed 100/0 showed an increase in spleen weight compared with those fed 0/100 and greater heart dry matter than piglets fed the 50/50 diet. Phosphorus concentration in the spleen was higher in piglets that received 25/75 when compared with those that received the 100/0 and 0/100 diets. Piglets that received the 0/100 diet showed higher pH of the stomach contents, but showed reduced pH of the contents of the jejunum and ileum. The width of the epiphysis and diaphysis was greater when piglets consumed the 50/50 treatment; however, a lower epiphysis height was observed. The use of $1,25(OH)_2D_3$ glycoside alone in piglet diets does not negatively influence the pH of the gastrointestinal tract and organ physicochemical composition. In addition, the use of diets with $1,25(OH)_2D_3$ glycoside as a replacement for cholecalciferol reduces spleen weight and promotes improvements in bone development.

Keywords: bone morphometry, organ parameters, piglet nutrition, *Solanum glaucophyllum*, vitamin D, weaned pigs

***Corresponding author:**
paulolevi@yahoo.com.br

Received: May 17, 2021
Accepted: September 29, 2021

How to cite: Trautenmüller, H.; Genova, J. L.; Faria, A. B. B.; Martins, J. S.; Viana, S. C. M.; Castilha, L. D.; Gonçalves Junior, A. C.; Baraldi-Artoni, S. M. and Carvalho, P. L. O. 2021. Bone traits and gastrointestinal tract parameters of piglets fed cholecalciferol and 1,25-dihydroxycholecalciferol glycoside. Revista Brasileira de Zootecnia 50:e20210098. https://doi.org/10.37496/rbz5020210098

1. Introduction

The metabolic system of vitamin D is primarily known to be essential for mineral homeostasis and bone metabolism. Calcitriol [$1,25(OH)_2D_3$] increases the intestinal absorption and renal reabsorption of calcium (Ca^{2+}) and phosphorus (P). These effects are mediated by VDR (vitamin D receptor) and regulate the expression of several genes by the binding to the vitamin D responsive element (VDRE) (Bergwitz and Jüppner, 2010; Haussler et al., 2011). Therefore, a diet with low Ca^{2+} concentration

will promote an increase in the conversion of vitamin D into its active form (Pérez et al., 2008; Bronner, 2003; Wasserman, 2004).

Among its non-calcemic functions, $1,25(OH)_2D_3$ has the ability to regulate innate and adaptive immunity, potentiating the innate response and suppressing adaptive immunity (Nagpal et al., 2005). Other actions of $1,25(OH)_2D_3$ is the effect of VDR suppression present in immune cells, preventing an excessive response by the immune system by reducing spleen weight (Hewison, 2012). The intracrine production of vitamin D by immune system cells in the presence of the enzyme 1α-hydroxylase stimulates the synthesis of antimicrobial peptides such as catelicidins, which contribute to the initial defense against pathogens and infections (Cantorna, 2010; White, 2010).

The segments of the gastrointestinal tract are relevant for calcium absorption, as is the pH at this site (González-Vega et al., 2014). In addition, dietary calcium and phosphorus content have been reported to directly affect their concentrations in several organs and tissues (González-Vega et al., 2016), with activation of 1,25-hydroxylase activity and an elevation in vitamin D_3 concentrations (Becker et al., 2020).

The plants of the Solanaceae family have a glycoside analogous to $1,25(OH)_2D_3$. This glycoside is broken down by bacteria with β-glycosidase activity present in the colon of monogastric animals and then absorbed. This metabolite is extracted from the plant *Solanum glaucophyllum* and can be used as a form of vitamin D supplementation. The main pharmacokinetic difference between the $1,25(OH)_2D_3$ glycoside and the synthetic $1,25(OH)_2D_3$ is the absorption speed, since the glycosidic form needs to be broken down to be absorbed. This mechanism causes a delay in absorption and a lower metabolic plasma peak and, consequently, a longer biological half-life, presenting a lower risk of toxicity (Zimmerman et al., 2015; Mathis et al., 2016).

Thus, vitamin D has the ability to regulate bone development (Schlegel et al., 2017). Although several studies have been conducted to assess the effects of vitamin D, more information is needed to investigate the different forms of vitamin D on gastrointestinal tract and organ function parameters. Therefore, the aim of this study was to assess the supplementation of two forms of vitamin D, cholecalciferol (vitamin D_3) and 1,25-dihydroxycholecalciferol glycoside ($1,25(OH)_2D_3$), alone or combined, in piglet feed on organ biometry (digestive and non-digestive organs) and physicochemical composition (heart, spleen, kidneys, liver, and bone), pH of the digestive tract contents, and bone traits.

2. Material and Methods

The study was conducted on an experimental farm located in Marechal Cândido Rondon, Paraná, Brazil (24°31'52" S and 54°01'03" W). Piglets were carefully managed to avoid unnecessary discomfort and all experimental procedures were approved by the local Research Ethics Committee (case no. 16/19). All procedures of euthanasia for the animals were performed by electronarcosis, in compliance with the Normative Resolution No. 37 of February 15, 2018 of CONCEA, which establishes the Guidelines of the Practice of Euthanasia of the National Council for Control of Animal Experimentation.

2.1. Animals and housing

A total of 128 entire hybrid male piglets (Landrace × Large White, Agroceres♂ and DanBred♀), weaned at 21 days-old and with an average initial body weight of 6.82±0.38 kg, were allocated in a randomized block design with eight replications and four animals per experimental unit. The blocks were formed based on the animals' initial body weight (Rinnert digital scale, model BPW-5000; Braço do Trombudo, SC, Brazil).

The animals were weighed, identified with numbered ear tags, and housed in a nursery facility at the beginning of experimental period. The suspended pens (1.54 m^2) were made of polyethylene plastic flooring, equipped with nipple drinking fountains and gutter feeders, arranged in two rows and divided by a central aisle. The experiment lasted 42 days.

The windows on the east and southeast side of the facility received black tarps to prevent the entry of intense solar radiation to prevent the animals' metabolism from converting the cholesterol into vitamin D; therefore, the only source of this vitamin was provided through the diet. All animals received light at the same intensity and duration during the experiment.

Air temperature and relative humidity were measured with datalogger equipment (UNI-T UT 330B digital USB). The temperature ranged from 19.5 to 33.1 °C with an average of 26.2 °C and relative humidity was between 30 and 90% during the experimental period. The nursery facility was ventilated with fans, exhaust fan, and tilting-type windows. The heating of the experimental pens was controlled by individual infrared incandescent lamps.

2.2. Experimental diets

The diets were formulated to meet nutritional requirements, following ranges close to those of the recommendations of Rostagno et al. (2017). The vitamin complex was free of vitamin D (Table 1). Four experimental treatments were tested, with different forms of supplementation of vitamin D, using cholecalciferol and 1,25$(OH)_2D_3$ glycoside. The sources of 1,25$(OH)_2D_3$ glycoside used were the dry leaves of the *Solanum glaucophyllum* plant (Panbonis®, Herbonis Animal Health), which provided 10 mg of 1,25$(OH)_2D_3$ per kg of product, according to analytical determination. The recommendation for using the product was 50 g t^{-1}, which was equivalent to 0.5 µg of 1,25$(OH)_2D_3$ per kg of diet.

The experimental treatments consisted of a diet based on: 100/0 = 100% of the vitamin D requirement supplemented with cholecalciferol (2707 IU in the pre-starter I, 2405 IU in the pre-starter II, and 1969 IU in the starter phase); 50/50 = 50% of the requirement supplemented with cholecalciferol + 0.25 µg of 1,25$(OH)_2D_3$ glycoside; 25/75 = 25% of the cholecalciferol requirement for each phase + 0.375 µg of 1,25$(OH)_2D_3$ glycoside; and 0/100 = 0.50 µg of 1,25$(OH)_2D_3$ glycoside.

The feeds were in powder form and produced by tumbling, using a Y-type mixer, with an uninterrupted mixing time of 12 min to ensure optimal mixing. After mixing each treatment, ground corn was used to clean up and minimize the residual effect of vitamin D between treatments.

2.3. Sample collection and preparation

At the end of the experimental period (42 days), 24 animals (six replications for each treatment) were selected for slaughter for the harvest of biological samples. The animals fasted for 12 h before being slaughtered. The pH of the stomach and intestine contents was measured *in loco* with the aid of a digital pH meter (Hanna Instruments Inc., Rhodes Island, USA, model HI 99163; Smithfield, RI, United States of America) immediately after slaughter. The relative organ weight (heart, liver, kidneys, intestines, and spleen) was calculated as the proportion of each organ relative to the body weight of slaughtered animal, using the equation: relative organ weight = (organ weight/body weight at slaughter) × 100.

The legs of slaughtered piglets were collected, packed in identified bags, and stored in a freezer. The samples were autoclaved at 120 °C and 1 atm for 10 min to remove the meat, and clean bones were refrozen. The third metacarpal of the right leg was used for bone densitometry analysis (Hologic Discovery Wi® software). Bone strength was performed in a Universal Mechanical Testing Machine (EMIC brand, DL 10,000 model, with an EMIC load-cell of 200 kgf), following recommendations of the standard (ANSI/ASAE S459 MAR 98) for the three-point flexion test with speed of 5 mm/min, 500 N preload with 30s accommodation time, and a distance of 60 mm between points. The data collected by a computer attached directly to the machine was expressed in Newtons (N) and then transformed into kilogram-force per square centimeter (kgf/cm^2).

The second metacarpal of the right leg was used to make slides, being first decalcified with a solution of formic acid (50%) and sodium citrate (20%) for 25 days. Thus, the slides were made, stained with

Table 1 - Centesimal and calculated composition of experimental diets offered to piglets in the experiment (%, as-fed basis)

Item	Pre-starter I	Pre-starter II	Starter
Ingredient (%)			
Ground corn (7.88%)	49.88	55.51	57.85
Soybean meal (45%)	17.47	19.90	30.52
Whey concentrate	11.36	8.00	-
Milk powder	4.00	-	-
Micronized soybean (36%)	8.00	8.00	4.00
Common sugar	4.00	3.00	2.00
Soybean oil[1]	1.53	2.01	2.42
Monocalcic phosphate	1.35	1.55	1.69
Calcitic limestone	0.19	0.37	0.40
Cooking salt	0.47	0.50	0.48
Citric acid	0.40	-	-
L-lysine HCl (78%)	0.40	0.42	0.19
L-threonin (98%)	0.14	0.14	0.03
L-tryptophan (98%)	0.08	0.08	0.04
DL-methionine (99%)	0.18	0.17	0.09
Premix[2]	0.20	0.20	0.20
Additive[3]	0.20	-	-
Growth promoter[4]	0.15	0.15	0.05
Copper sulfate (25%)	-	-	0.04
Total	100	100	100
Calculated composition (%)			
ME (kcal/kg)	3.50	3.45	3.40
Ether extract	6.39	6.07	5.77
Total calcium	0.50	0.55	0.55
Available phosphorus	0.45	0.45	0.45
Digestible lysine	1.16	1.15	1.10
Digestible methionine + cystine	0.68	0.68	0.67
Digestible threonine	0.73	0.72	0.69
Digestible tryptophan	0.24	0.23	0.21
Total sodium	0.30	0.27	0.21
Lactose	10.00	6.00	-
Analysed composition (%NM)			
Dry matter	95.15	95.27	95.41
Crude protein	20.84	20.89	23.52
Mineral matter	4.71	4.63	4.96

NM - natural matter.
[1] Refined vegetable oil: pre-starter I; degummed vegetable oil: pre-starter II and starter.
[2] Vitamin and mineral premix: pre-starter I and II phases = vitamin A (min.), 3,850,000 IU/kg; vitamin E (min.), 22,400 IU/kg; vitamin K_3 (min.), 1,680 IU/kg; vitamin B_1 (min.), 560 mg/kg; vitamin B_2 (min.), 1,750 mg/kg; vitamin B_6 (min.), 1,120 mg/kg; vitamin B_{12} (min.), 11,000 mcg/kg; niacin (min.), 17 g/kg; pantothenic acid (min.), 8,400 mg/kg; folic acid (min.), 168 mg/kg; biotin (min.), 56 mg/kg; choline (min.), 112 g/kg; manganese (min.), 22.50 g/kg; zinc (min.), 61.50 g/kg; iron (min.), 45 g/kg; copper (min.), 6,700 mg/kg; iodine (min.), 560 mg/kg; selenium (min.), 205 mg/kg. Starter phase = vitamin A (min.), 3,437,500 IU/kg; vitamin E (min.), 20,000 IU/kg; vitamin K_3 (min.), 1,500 IU/kg; vitamin B_1 (min.), 500 mg/kg; vitamin B_2 (min.), 1,565 mg/kg; vitamin B_6 (min.), 1,000 mg/kg; vitamin B_{12} (min.), 10,000 mcg/kg; niacin (min.), 15 g/kg; pantothenic acid (min.), 7,500 mg/kg; folic acid (min.), 150 mg/kg; biotin (min.), 50 mg/kg; choline (min.), 100 g/kg; manganese (min.), 20 g/kg; zinc (min.), 55 g/kg; iron (min.), 40 g/kg; copper (min.), 6,000 mg/kg; iodine (min.), 500 mg/kg; selenium (min.), 180 mg/kg.
[3] Nutritional additive, source of free and purified nucleotides.
[4] Colistin, 8%.

hematoxylin and eosin, as well as photomicrographed on an optical microscope with a built-in camera; measurements of the growth plates were made using Image-Pro Plus analysis software.

The third metatarsal was used for external morphological measurements, with a digital caliper (Lorben brand, with measurement from 0.01 to 150 mm). Measurements were made of the entire bone (epiphysis to epiphysis), epiphysis length (tip of epiphysis to growth plate), epiphysis width (middle of epiphysis), and diaphysis width (middle of bone). Weight was measured using a semi-analytical scale. The Seedor index was calculated by dividing bone weight (mg) by its length (mm) (Seedor et al., 1991).

The second and third left metacarpals were dried in an oven (Tecnal brand, SF-325 NM model; Piracicaba, SP, Brazil) at 65 °C for 72 h and then degreased, using petroleum ether for four days, with the solution being renewed on the second day. Afterwards, ether was removed, and bones were dried for 24 h in an oven at 65 °C (Tecnal brand, SF-325 NM model; Piracicaba, SP, Brazil), ground in a closed-chamber ball mil to perform the analysis of dry matter in an oven at 105 °C (Tecnal brand, TE 393/2 model; Piracicaba, SP, Brazil) and of mineral matter using the muffle furnace (Fornitec brand, F2 DM single-phase model; São Paulo, SP, Brazil) at 600 °C for 4 h.

2.4. Statistical analysis and calculations

Data were evaluated by analyzing standardized Student residues to find outliers, then covariance (ANCOVA) and variance (ANOVA) were performed. Normality of experimental errors and homogeneity of variances between treatments for the several variables were previously assessed using the Shapiro-Wilk and Levene tests, respectively.

For the characteristics of relative organ weight and bone morphometry, the statistical model used was:

$$Y_{ijk} = m + T_i + b_j + \beta (X_{ijk} - \bar{X}...) + \varepsilon_{ijk},$$

in which Y_{ijk} = average observation of the dependent variable in each plot, measured in the *i*-th treatment class, in the *j*-th block, and in the *k*-th replication; m = effect of the general average; T_i = effect of treatment classes, for *i* = (1, 2, 3, and 4); b_j = block effect, for *j* = (1 and 2); β = regression coefficient of Y over X; X_{ijk} = average observation of the covariate (weight of digestive and non-digestive organs and slaughter weight of the animals) in each plot, measured in the *i*-th treatment class, in the *j*-th block, and in the *k*-th replication; $\bar{X}...$ = general average for the covariate X; and ε_{ijk} = random plot error associated with *i*-th level, *j*-th block, and *k*-th replication.

For the pH characteristics of the digestive tract content, organ chemical composition, and bone strength and density, the statistical model used was the one mentioned above, without including the covariate effect.

Comparisons between treatment averages were performed using Tukey's test at the level of 5 to 10% probability. The β error of the parameters was used to help explain the P-values of 5 to 10% probability. This procedure was performed using the test power (1-β) of the R package. Statistical analyses were performed using the procedures of the statistical software SAS (Statistical Analysis System, University Edition). All data were presented as averages with standard error of the mean.

3. Results

3.1. Weight and physicochemical composition of organs

The results indicated that piglets fed 100/0 showed (P = 0.032) an increase in spleen weight compared with those fed 0/100 (Table 2) and greater (P = 0.061) heart dry matter than piglets fed 50/50 diets (Table 3). In the physicochemical composition, phosphorus concentration in the spleen was higher (P = 0.048) in piglets that received 25/75 when compared with those that received the 100/0 and 0/100 treatments (Table 3).

Table 2 - Relative weight of digestive and non-digestive organs (% of body weight) of piglets fed cholecalciferol and 1,25(OH)$_2$D$_3$ glycoside

	Treatment[1]				Mean	SEM	P-value
	100/0	50/50	25/75	0/100			
Heart	0.51	0.56	0.53	0.51	0.53	0.011	0.135
Kidneys	0.55	0.58	0.55	0.54	0.55	0.013	0.367
Liver + gallbladder	3.10	2.97	3.07	2.97	3.03	0.066	0.797
Empty stomach	0.83	0.82	0.79	0.75	0.80	0.029	0.523
Spleen	0.22a	0.18ab	0.18ab	0.17b	0.19	0.007	0.032
SI and pancreas	3.97	3.50	3.32	4.20	3.75	0.184	0.354
Empty large intestine	2.80	2.56	2.96	2.57	2.72	0.131	0.725
Empty SI (m)	12.80	13.35	12.25	12.58	12.74	0.284	0.713

SI - small intestine.
[1] Experimental diets: 100/0 = 100% cholecalciferol; 50/50 = 50% cholecalciferol + 50% 1,25(OH)$_2$D$_3$ glycoside; 25/75 = 25% cholecalciferol + 75% 1,25(OH)$_2$D$_3$ glycoside; 0/100 = 100% 1,25(OH)$_2$D$_3$ glycoside.
a,b - Values followed by different letters in the row differ from each other according to Tukey's test at the 10% probability level.

Table 3 - Organ physicochemical composition of piglets fed partial or total replacement of cholecalciferol by 1,25(OH)$_2$D$_3$ glycoside

	Treatment[1]				Mean	SEM	P-value
	100/0	50/50	25/75	0/100			
				Dry matter (%)			
Heart	20.30a	19.00b	19.61ab	19.42ab	19.58	0.175	0.061
Spleen	19.10	19.23	18.93	18.88	19.04	0.108	0.699
Kidneys	17.47	17.72	17.11	17.45	17.44	0.147	0.570
Liver	26.24	25.77	26.56	25.83	26.10	0.230	0.643
				Mineral matter (%)			
Heart	12.07	12.42	11.25	8.57	11.08	0.721	0.245
Spleen	11.10	13.51	10.11	10.51	11.30	0.674	0.315
Kidneys	10.06	9.70	9.13	8.75	9.41	0.306	0.482
Liver	12.46	10.68	9.53	12.81	11.37	0.818	0.344
Bone	48.31	49.03	48.82	47.95	48.53	0.393	0.721
				Calcium (g/kg)			
Heart	1.07	0.90	0.83	0.90	0.92	0.051	0.363
Spleen	0.70	0.82	0.96	0.93	0.85	0.059	0.451
Kidneys	0.90	0.83	0.90	1.09	0.93	0.043	0.194
Liver	0.70	0.78	0.75	0.81	0.76	0.037	0.796
Bone	167.10	159.36	157.13	159.90	160.87	2.954	0.666
				Phosphorus (g/kg)			
Heart	53.87	59.18	54.15	59.25	56.61	1.179	0.198
Spleen	66.64b	75.54ab	77.03a	66.51b	71.43	1.765	0.048
Kidneys	74.49	75.71	76.30	77.70	76.05	0.962	0.702
Liver	73.62	76.88	75.05	75.69	75.31	1.005	0.729
Bone	216.40	215.69	216.50	216.54	216.28	0.167	0.232

[1] Experimental diets: 100/0 = 100% cholecalciferol; 50/50 = 50% cholecalciferol + 50% 1,25(OH)$_2$D$_3$ glycoside; 25/75 = 25% cholecalciferol + 75% 1,25(OH)$_2$D$_3$ glycoside; 0/100 = 100% 1,25(OH)$_2$D$_3$ glycoside.
a,b - Values followed by different letters in the row differ from each other according to Tukey's test at 10% probability level.

3.2. pH of digestive tract contents

The pH of the stomach contents demonstrated an effect (P = 0.076) with a reduction in piglets in the 50/50 treatment when compared with those in the 100/0 and 0/100 treatments. Piglets that

received 50/50-based diets showed higher (P = 0.000) pH of the jejunum contents than those fed the 25/75 and 0/100 diets. However, the pH of the ileal content was lower (P = 0.002) in piglets fed the 0/100 diet (Table 4).

Table 4 - pH values of the gastrointestinal contents of piglets fed partial or total replacement of cholecalciferol by $1,25(OH)_2D_3$ glycoside

	Treatment[1]				Mean	SEM	P-value	1-β (%)[2]
	100/0	50/50	25/75	0/100				
Stomach	3.30a	1.89b	2.71ab	3.37a	2.82	0.244	0.076	72.683
Duodenum	5.23	6.00	4.89	5.18	5.32	0.214	0.240	46.468
Jejunum	6.60ab	6.69a	6.42bc	6.37c	6.52	0.035	0.000	99.885
Ileum	6.53a	6.68a	6.55a	6.07b	6.45	0.069	0.002	99.391
Cecum	5.63	5.61	5.69	5.47	5.60	0.069	0.766	14.101
Colon	5.63	5.96	5.81	5.64	5.76	0.063	0.233	47.438

[1] Experimental diets: 100/0 = 100% cholecalciferol; 50/50 = 50% cholecalciferol + 50% $1,25(OH)_2D_3$ glycoside; 25/75 = 25% cholecalciferol + 75% $1,25(OH)_2D_3$ glycoside; 0/100 = 100% $1,25(OH)_2D_3$ glycoside.
[2] 1-β (%) = statistical test considering alpha 5%.
a-c - Values followed by different letters in the row differ from each other according to Tukey's test at 10% probability level.

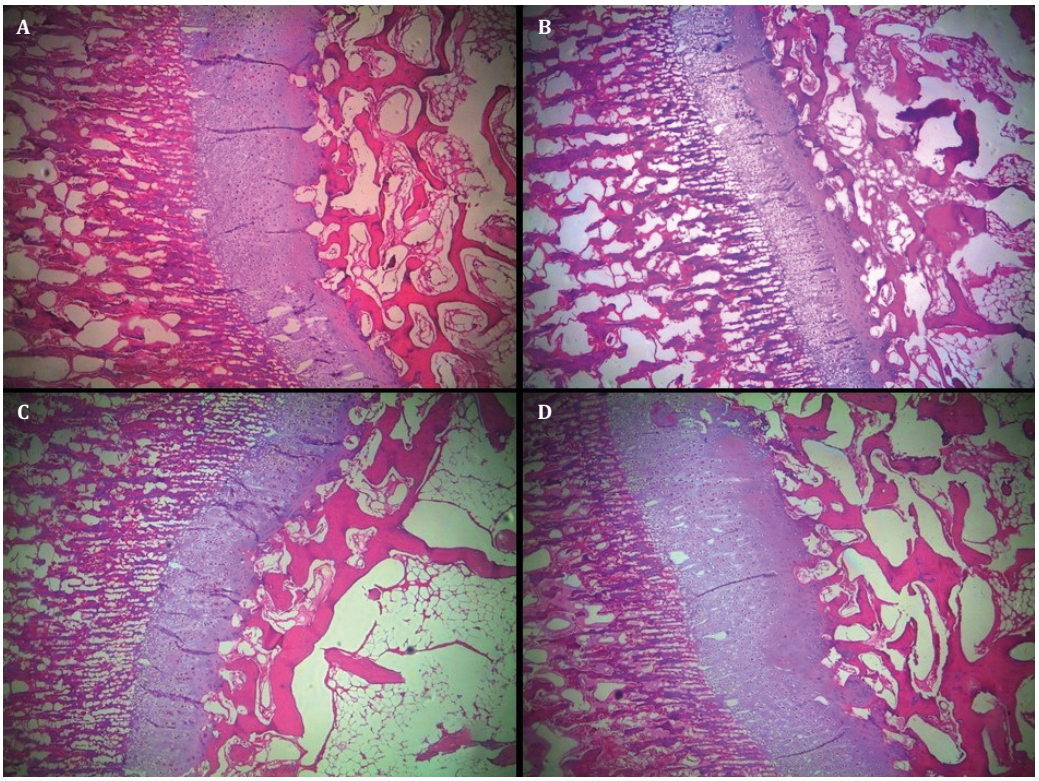
3.3. Bone traits

Through bone morphometry of the metatarsal bone, a difference (P = 0.013) was verified for the parameter epiphysis width, in which piglets that received 50/50 diet showed higher average values compared with those that received the 100/0 diet. A similar effect (P = 0.049) was observed for diaphysis width, with piglets fed the 50/50 or 0/100 diet showing the highest averages values, and the opposite was found (P = 0.068) for diaphysis height (Table 5). Metacarpal bone strength and density showed no differences (P>0.1) between treatments. Piglets fed only $1,25(OH)_2D_3$ glycoside showed a higher (P = 0.002) growth plate than piglets that partially ingested cholecalciferol in their diets, showing an increase in bone cell proliferation (Figure 1).

Table 5 - Bone traits of metatarsals and metacarpals of piglets fed partial or total replacement of cholecalciferol by $1,25(OH)_2D_3$ glycoside

	Treatment[1]				Mean	SEM	P-value
	100/0	50/50	25/75	0/100			
				Metatarsal bone morphometry (mm)			
Epiphysis height	20.46	25.98	20.93	25.62	23.25	1.326	0.292
Epiphysis width	17.84b	27.04a	19.06ab	25.06ab	22.23	1.256	0.013
Diaphysis height	41.12a	37.93b	39.52ab	37.23b	38.95	0.557	0.068
Diaphysis width	15.77b	22.60a	16.49ab	22.41a	19.32	1.166	0.049
Total length	61.58	65.26	60.46	64.33	62.91	1.455	0.608
Growth plate	6.84ab	5.81bc	5.15c	7.56a	6.34	0.277	0.002
Wet weight (g)	9.43	9.77	10.17	10.54	9.98	0.491	0.554
				Metacarpal bone strength and density			
Bone area (cm^2)	4.91	5.07	5.07	4.89	4.98	0.132	0.945
BMC (g)	0.83	0.91	0.91	0.79	0.86	0.031	0.507
BMD (g/cm^2)	0.16	0.17	0.18	0.16	0.17	0.003	0.353
Strength (kgf)	21.36	30.51	27.96	27.68	26.88	2.040	0.459
Strength (N)	209.49	299.20	274.24	271.54	263.61	20.011	0.459
Seedor's index	208.18	200.06	211.36	222.55	210.54	6.483	0.340

BMC - bone mineral content; BMD - bone mineral density.
[1] Experimental diets: 100/0 = 100% cholecalciferol; 50/50 = 50% cholecalciferol + 50% $1,25(OH)_2D_3$ glycoside; 25/75 = 25% cholecalciferol + 75% $1,25(OH)_2D_3$ glycoside; 0/100 = 100% $1,25(OH)_2D_3$ glycoside.
a-c - Values followed by different letters in the row differ from each other according to Tukey's test at 10% probability level.



100/0 = 100% of the vitamin D supplemented with cholecalciferol (A); 50/50 = 50% supplemented with cholecalciferol + 0.25 µg of $1,25(OH)_2D_3$ glycoside (B); 25/75 = 25% supplemented with cholecalciferol + 0.375 µg of $1,25(OH)_2D_3$ glycoside (C); 0/100 = 0.50 µg of $1,25(OH)_2D_3$ glycoside (D).

Figure 1 - Photomicrograph of histological slides of the growth plate from the second metacarpal bone of piglets (eosin-hematoxylin staining, 10X magnification).

4. Discussion

4.1. Weight and physicochemical composition of organs

The greater relative weight of the spleen in the cholecalciferol treatment may be associated with a general stimulus in the piglet's immune system (Smith and Hunt, 2004). Immune cells, such as macrophages, neutrophils, lymphocytes, and antigen-presenting cells, produce the enzyme 1alpha-hydroxylase (Nelson et al., 2010). This enzyme is stimulated by the activation of toll-like receptors (TLR), which are activated when they recognize molecular patterns of pathogenic microorganisms such as peptideoglycans, lipopolysaccharides, and lipopeptides. Activation of the vitamin D-mediated immune response occurs by converting $25(OH)D_3$ to $1,25(OH)_2D_3$ in the macrophage cytosol; therefore, if the calcidiol level is low, the response may be compromised (Andrade et al., 2011).

As the adaptive immunity is suppressed by $1,25(OH)_2D_3$, there is a reduction in the activity of immune cells, and production of lymphocytes by the spleen is reduced; therefore, atrophy or reduction in the organ size can occur, as found in piglets that received the treatment based only on $1,25(OH)_2D_3$ glycoside. Despite the difference found in the relative spleen weight, they are within the normal range reported in the literature for pigs at this phase (Pozza et al., 2010; Andrade et al., 2011).

The conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ by 1α-hydroxylase occurs in keratinocytes and monocytes through the mediation of TLR. In the presence of infections, the activation of these receptors promotes an increase in the expression of 1alpha-amylase enzyme (CYP27B1), resulting in conversion of active vitamin D with the release of catelicidin, an antimicrobial peptide (Liu et al., 2006;

Hata et al., 2008). Therefore, it is understood that there is a need for conversion of vitamin D from calcidiol to calcitriol for the occurrence of immunological effects. When only $1,25(OH)_2D_3$ glycoside was provided in the diet, there was little or no conversion of vitamin D from the diet, resulting in smaller spleen size in piglets that consumed the $1,25(OH)_2D_3$ glycoside treatment.

Similar values for the variables analyzed in the organs showed a mineral balance in the animals' organism. Regardless of the source of vitamin D used, the levels of mineral matter, calcium, and phosphorus did not change, except for phosphorus in the spleen. This mineral homeostasis demonstrates a regulated control in the production, inactivation of $1,25(OH)_2D_3$, and control in the metabolism of these minerals.

The spleen is one of the organs with the highest concentration of phosphorus in its composition, as well as the brain, liver, and muscles. The absorption of this mineral can vary according to the animal's age, molecular structure, calcium, phosphorus, intestinal pH, and dietary levels. There is no known activity of vitamin D on the deposition of this mineral in the spleen; therefore, the difference in phosphorus concentration in this organ may have been caused by several factors, since phosphorus is one of the most abundant elements in the body, used in metabolic reactions and as an energy source (Georgievskii, 1982; Teixeira et al., 2004; Stein et al., 2011).

The higher concentration of calcium and phosphorus in the bones compared with the organs suggests that the animals received sufficient supplementation of these minerals in all treatments, with formation of hydroxyapatite in the bones as a form of mineral reserve. The exchange in tissues decreases with age and increases during reproductive periods with more intense exchanges between the bone and the bloodstream (Figueiredo et al., 2001; Moreira et al., 2004).

In a study conducted by Witschi et al. (2011), the metabolite $25(OH)_2D_3$ was used in diet of piglets in nursery phase and, compared with cholecalciferol, no differences were found in calcium and phosphorus levels in the bones, as well as in mineral matter between treatments using both forms of vitamin D. The authors found average mineral matter values of 37.56% in bone, while in the present study, higher values were found with an average of 48.53%. Phosphorus is found in many body tissues, and the incorporation of this mineral into tissues and organs can vary depending on the rate of renewal and growth phase of the animal (Moreira et al., 2004).

The phosphorus content measured in bones was greater than the content of calcium, which is not found in the literature, since the calcium requeriment in bones is higher than the phosphate due to the constitution of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$); therefore, ten molecules of calcium and six of phosphate are required for their formation. Results found in the literature demonstrate a Ca:P ratio in the bones of about 2.2:1, corroborating the value required for the formation of hydroxyapatite. Otherwise, the Ca:P ratio found by the average of treatments was 1:1.3 in the present study (O'Doherty et al., 2010; Zhou et al., 2017; Duffy et al., 2018). However, the dietary calcium content was based on commercial diets, with formulated values below the nutritional requirements (NRC, 2012; Rostagno et al., 2017), promoting different concentration values in the bone. An increase in dietary phosphorus content has limited effect on the phosphorus content in soft tissue, whereas the phosphorus retention in the bone is enhanced (Lautrou et al., 2020). In this case, bones are the default storage site for calcium and phosphorus if availability exceeds growth requirement (González-Vega et al., 2016).

4.2. pH of digestive tract contents

Changes in digesta pH levels are influenced by several factors such as nutritional composition and feed intake (González-Vega et al., 2014), health status, environmental/housing condition (Jayaraman and Nyachoti, 2017), and measurement site (Heo et al., 2013).

The lowest pH values were found in treatments with the combination of the two forms of vitamin D [cholecalciferol and $1,25(OH)_2D_3$ glycoside]. The stomach pH is important to start the digestion process, form a barrier against pathogens, and maintain a suitable environment for the activation of

enzymes such as pepsin, which is responsible for protein digestion. The ideal pH for these functions to be performed is between 2.0 and 3.5, and high values can contribute to the proliferation of bacteria such as *Escherichia coli* and *Salmonella* spp. (Gonzales et al., 2013). According to Heo et al. (2013), optimal pH for dietary protein digestion in the stomach is 3.0, which is close to the values found in the present study.

Low pH activates pepsin within the stomach, as well as the dissociation of compounds formed by calcium, releasing Ca^{2+} ions for intestinal absorption. Most calcium absorption occurs in the small intestine, mainly in the duodenum and jejunum (Schröder and Breves, 2006; Corassa et al., 2012). The lower pH of the jejunum for treatment with the $1,25(OH)_2D_3$ glycoside may have influenced calcium absorption at this site, and a higher digesta pH has been attributed to reduced calcium and phosphorus absorption in pigs (González-Vega et al., 2014).

In newborn piglets, the main stomach acidifier is lactic acid produced by lactose fermentation. The production of hydrochloric acid (HCl) gradually increases from the fourth to the eighth week of life. However, its efficiency is not at maximum potential, causing a higher pH than desired; hence, there is a need to use acidifiers in piglet diet (Denck et al., 2017).

The use of citric acid as an acidifier in the experimental diet of the pre-starter I phase may have caused an increase in HCl production with a consequent reduction in stomach pH. Fernandes and Miranda (2013) reported that the increase of endogenously-produced organic acids have the ability to reduce intestinal pH according to the ingredients used in piglet diets.

The results of this experiment suggest that variation between the vitamin D sources studied may result in differences in calcium absorption site. González-Vega et al. (2014) reported that calcium can be absorbed in the stomach or in the early portion of the duodenum depending on the calcium source and feed intake, influencing the pH of the gastrointestinal tract.

4.3. Bone traits

Osteochondrosis lesions in pigs are most commonly found in the humerus and femur, but can also occur in the shoulder, hip, ischial joints, vertebral joints, and costochondral junctions of ribs. These lesions can be observed in histological analysis of two-month-old piglets; however, the animals present the so-called "leg weakness" when they reach greater body weight (Ytrehus et al., 2007). The present study did not aim to assess the incidence of osteochondrosis, but improved bone development may contribute to the reduction of these problems.

Bone morphometry analysis showed that with the exclusive supply of $1,25(OH)_2D_3$ glycoside in diets, there was a greater diaphysis width, but reduced length, and the opposite was found in the cholecalciferol treatment. However, epiphysis height was not different, and epiphysis width was smaller with the use of cholecalciferol. During bone formation, there is growth through the zone chondrocyte proliferation in the growth plate. This multiplication of chondrocytes is what gives rise to the increase in bone size in length (Wongdee et al., 2012). Although the piglets that received $1,25(OH)_2D_3$ glycoside had the shortest diaphysis length, there was no difference in total bone length among treatments, and the glycoside compound treatment showed the greater growth plate.

Amundson et al. (2016) found higher bone mineral density (BMD) values in the bones of eight-week-old piglets when cholecalciferol supplementation was used with a 20% increase in the animals' phosphorus requirement (0.430 g/cm^2) and higher values when sows were supplemented with cholecalciferol during pregnancy and lactation (0.514 g/cm^2). The influence of minerals may have contributed to the differences found in this investigation, as calcemia and phosphatemia are important regulators of the conversion of vitamin D and mineral deposition in bones.

Castro et al. (2018) found no effect on bone strength in poultry fed cholecalciferol and synthetic $1,25(OH)_2D_3$; however, when 100% of the cholecalciferol recommendation was used, it showed a higher BMD than the treatment that used only 50%, demonstrating the need for adequate supplementation

of this vitamin to improve BMD. The highest BMD value was when 100% of the recommendation was used associated with $1,25(OH)_2D_3$ supplementation.

In a recent study, Schlegel et al. (2017) used cholecalciferol alone or combined with increasing levels of $1,25(OH)_2D_3$ glycoside in piglet feeding and found differences between treatments on tibial strength, in which the highest level of glycoside supplementation presented similar strength to treatment with cholecalciferol alone; however, density was not different between treatments.

The development of the growth plate with the use of $1,25(OH)_2D_3$ glycoside may be related to the function of the active form in calcium and phosphorus metabolism, acting directly on the growth plate chondrocytes that express CYP27B1, an enzyme that converts $25(OH)_2D_3$ into $1,25(OH)_2D_3$. Vitamin D active at this site has autocrine functions, such as cell differentiation, angiogenesis, and osteoclastogenesis (Naja et al., 2009).

The genomic action of $1,25(OH)_2D_3$ regulates bone development and phosphate homeostasis by paracrine. These regulated genes are essential for vascularization and invasion of osteoclasts into the area of hypertrophic chondrocytes and indirectly act on development of the growth plate (Masuyama et al., 2006).

Periods of rapid growth are likely indications of unbalanced skeletal growth. Thus, the period from weaning to 84 days of age is called the "window of susceptibility", when mature cartilage is most vulnerable to the onset of osteochondrotic lesions. Pigs that showed rapid growth between 28 and 35 days old had severe osteochondrosis at slaughter age, and between 56 and 84 days old, they had mild osteochondrosis; however, after 90 days old, body weight gain was not related to the onset of osteochondrosis (van Grevenhof et al., 2012).

5. Conclusions

The use of $1,25(OH)_2D_3$ glycoside alone in piglet diets does not negatively influence the pH of the gastrointestinal tract and organ physicochemical composition. The use of diets with $1,25(OH)_2D_3$ glycoside as a replacement for cholecalciferol reduces spleen weight and promotes improvements in bone development.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: H. Trautenmüller and P.L.O. Carvalho. Data curation: H. Trautenmüller, J.L. Genova, A.B.B. Faria, J.S. Martins, S.C.M. Viana, A.C. Gonçalves Junior, S.M. Baraldi-Artoni and P.L.O. Carvalho. Formal analysis: H. Trautenmüller, J.L. Genova, L.D. Castilha, A.C. Gonçalves Junior, S.M. Baraldi-Artoni and P.L.O. Carvalho. Funding acquisition: P.L.O. Carvalho. Investigation: H. Trautenmüller, J.L. Genova, A.B.B. Faria, J.S. Martins, S.C.M. Viana and P.L.O. Carvalho. Methodology: H. Trautenmüller, J.L. Genova, A.B.B. Faria, J.S. Martins, S.C.M. Viana, A.C. Gonçalves Junior, S.M. Baraldi-Artoni and P.L.O. Carvalho. Project administration: H. Trautenmüller and P.L.O. Carvalho. Resources: A.C. Gonçalves Junior, S.M. Baraldi-Artoni and P.L.O. Carvalho. Supervision: H. Trautenmüller and P.L.O. Carvalho. Validation: J.L. Genova, A.C. Gonçalves Junior and S.M. Baraldi-Artoni. Visualization: J.L. Genova, L.D. Castilha and P.L.O. Carvalho. Writing-original draft: H. Trautenmüller, J.L. Genova, L.D. Castilha and P.L.O. Carvalho. Writing-review & editing: H. Trautenmüller, J.L. Genova, L.D. Castilha and P.L.O. Carvalho.

Acknowledgments

The authors are grateful to the NutriQuest Technofeed for the suport in this research, and to the Cooperativa Agroindustrial Copagril.

References

Amundson, L. A.; Hernandez, L. L.; Laporta, J. and Crenshaw, T. D. 2016. Maternal dietary vitamin D carry-over alters offspring growth, skeletal mineralisation and tissue mRNA expression of genes related to vitamin D, calcium and phosphorus homoeostasis in swine. British Journal of Nutrition 116:774-787. https://doi.org/10.1017/S0007114516002658

Andrade, C.; Almeida, V. V.; Costa, L. B.; Berenchtein, B.; Mourão, G. B. and Miyada, V. S. 2011. Levedura hidrolisada como fonte de nucleotídeos para leitões recém-desmamados. Revista Brasileira de Zootecnia 40:788-796. https://doi.org/10.1590/S1516-35982011000400012

Becker, S. L.; Gould, S. A.; Petry, A. L.; Kellesvig, L. M. and Patience, J. F. 2020. Adverse effects on growth performance and bone development in nursery pigs fed diets marginally deficient in phosphorus with increasing calcium to available phosphorus ratios. Journal of Animal Science 98:skaa325. https://doi.org/10.1093/jas/skaa325

Bergwitz, C. and Jüppner, H. 2010. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annual Review of Medicine 61:91-104. https://doi.org/10.1146/annurev.med.051308.111339

Bronner, F. 2003. Mechanisms of intestinal calcium absorption. Journal of Cellular Biochemistry 88:387-393. https://doi.org/10.1002/jcb.10330

Cantorna, M. T. 2010. Mechanisms underlying the effect of vitamin D on the immune system. Proceedings of the Nutrition Society 69:286-289. https://doi.org/10.1017/S0029665110001722

Castro, F. L. S.; Baião, N. C.; Ecco, R.; Louzada, M. J. Q.; Melo, E. F.; Saldanha, M. M.; Triginelli, M. V. and Lara, L. J. C. 2018. Effects of 1,25-dihydroxycholecalciferol and reduced vitamin D_3 level on broiler performance and bone quality. Revista Brasileira de Zootecnia 47:e20170186. https://doi.org/10.1590/rbz4720170186

Corassa, A.; Lopes, D. C. and Bellaver, C. 2012. Mananoligossacarídeos, ácidos orgânicos e probióticos para leitões de 21 a 49 dias de idade. Archivos de Zootecnia 61:467-476. https://doi.org/10.4321/S0004-05922012000300015

Denck, F. M.; Hilgemberg, J. O. and Lehnen, C. R. 2017. Uso de acidificantes em dietas para leitões em desmame e creche. Archivos de Zootecnia 66:629-638. https://doi.org/10.21071/az.v66i256.2782

Duffy, S. K.; Kelly, A. K.; Rajauria, G.; Clarke, L. C.; Gath, V.; Monahan, F. J. and O'Doherty, J. V. 2018. The effect of 25-hydroxyvitamin D_3 and phytase inclusion on pig performance, bone parameters and pork quality in finisher pigs. Journal of Animal Physiology and Animal Nutrition 102:1296-1305. https://doi.org/10.1111/jpn.12939

Fernandes, A. and Miranda, A. P. 2013. Desempenho e ocorrência de diarreia em leitões alimentados com soro de leite. Archivos de Zootecnia 62:589-594. https://doi.org/10.4321/S0004-05922013000400011

Figueiredo, A. V.; Vitti, D. M. S. S.; Lopes, J. B. and Barbosa, H. P. 2001. Disponibilidade biológica do fósforo de fontes fosfatadas determinada por intermédio da técnica de diluição isotópica: II. Suínos em crescimento. Revista Brasileira de Zootecnia 30:1514-1520. https://doi.org/10.1590/S1516-35982001000600019

Georgievskii, V. I. 1982. The phisiological role of macroelements. p.91-170. In: Mineral nutrition of animals. Georgievskii, V. I.; Annenkov, B. N. and Samokhin, V. T., eds. Butterworths, London.

Gonzales, L.; Ali, Z. B.; Nygren, E.; Wang, Z.; Karlsson, S.; Zhu, B.; Quiding-Järbrink, M. and Sjöling, Å. 2013. Alkaline pH Is a signal for optimal production and secretion of the heat labile toxin, LT in enterotoxigenic *Escherichia coli* (ETEC). PLoS One 8:e74069. https://doi.org/10.1371/journal.pone.0074069

González-Vega, J. C.; Walk, C. L.; Liu, Y. and Stein, H. H. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Archives of Animal Nutrition 68:126-142. https://doi.org/10.1080/1745039X.2014.892249

González-Vega, J. C.; Walk, C. L.; Murphy, M. R. and Stein, H. H. 2016. Requirement for digestible calcium by 25 to 50 kg pigs at different dietary concentrations of phosphorus as indicated by growth performance, bone ash concentration, and calcium and phosphorus balances. Journal of Animal Science 94:5272-5285. https://doi.org/10.2527/jas.2016-0751

Haussler, M. R.; Jurutka, P. W.; Mizwicki, M. and Norman, A. W. 2011. Vitamin D receptor (VDR)-mediated actions of $1\alpha,25(OH)_2$vitamin D_3: genomic and non-genomic mechanisms. Best Practice and Research: Clinical Endocrinology and Metabolism 25:543-559. https://doi.org/10.1016/j.beem.2011.05.010

Hata, T. R.; Kotol, P.; Jackson, M.; Nguyen, M.; Paik, A.; Udall, D.; Kanada, K.; Yamasaki, K.; Alexandrescu, D. and Gallo, R. L. 2008. Administration of oral vitamin D induces cathelicidin production in atopic individuals. Journal of Allergy and Clinical Immunology 122:829-831. https://doi.org/10.1016/j.jaci.2008.08.020

Heo, J. M.; Opapeju, F. O.; Pluske, J. R.; Kim, J. C.; Hampson, D. J. and Nyachoti, C. M. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. Journal of Animal Physiology and Animal Nutrition 97:207-237. https://doi.org/10.1111/j.1439-0396.2012.01284.x

Hewison, M. 2012. Vitamin D and the immune system: new perspectives on an old theme. Rheumatic Disease Clinics of North America 38:125-139.

Jayaraman, B. and Nyachoti, C. M. 2017. Husbandry practices and gut health outcomes in weaned piglets: A review. Animal Nutrition 3:205-211. https://doi.org/10.1016/j.aninu.2017.06.002

Lautrou, M.; Pomar, C.; Dourmad, J. Y.; Narcy, A.; Schmidely, P. and Létourneau-Montminy, M. P. 2020. Phosphorus and calcium requirements for bone mineralisation of growing pigs predicted by mechanistic modelling. Animal 14:s313-s322. https://doi.org/10.1017/S1751731120001627

Liu, P. T.; Stenger, S.; Li, H.; Wenzel, L.; Tan, B. H.; Krutzik, S. R.; Ochoa, M. T.; Schauber, J.; Wu, K.; Meinken, C.; Kamen, D. L.; Wagner, M.; Bals, R.; Steinmeyer, A.; Zügel, U.; Gallo, R. L.; Eisenberg, D.; Hewison, M.; Hollis, B. W.; Adams, J. S.; Bloom, B. R. and Modlin, R. L. 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311:1770-1773. https://doi.org/10.1126/science.1123933

Masuyama, R.; Stockmans, I.; Torrekens, S.; Van Looveren, R.; Maes, C.; Carmeliet, P.; Bouillon, R. and Carmeliet, G. 2006. Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. Journal of Clinical Investigation 116:3150-3159. https://doi.org/10.1172/JCI29463

Mathis, G.; Boland, R.; Bachmann, H.; Toggenburger, A. and Rambeck, W. 2016. Safety profile of 1,25-dihydroxyvitamin D_3 of herbal origin in broiler chicken. Schweizer Archiv Fur Tierheilkunde 158:819-826. https://doi.org/10.17236/sat00097

Moreira, J. A.; Vitti, D. M. S. S.; Lopes, J. B. and Trindade Neto, M. A. 2004. Cinética do fósforo em tecidos de suínos alimentados com dietas contendo enzima fitase. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 56:74-80. https://doi.org/10.1590/S0102-09352004000100012

Nagpal, S.; Na, S. and Rathnachalam, R. 2005. Noncalcemic actions of vitamin D receptor ligands. Endocrine Reviews 26:662-687. https://doi.org/10.1210/er.2004-0002

Naja, R. P.; Dardenne, O.; Arabian, A. and St Arnaud, R. 2009. Chondrocyte-specific modulation of Cyp27b1 expression supports a role for local synthesis of 1,25-dihydroxyvitamin D_3 in growth plate development. Endocrinology 150:4024-4032. https://doi.org/10.1210/en.2008-1410

Nelson, C. D.; Reinhardt, T. A.; Beitz, D. C. and Lippolis, J. D. 2010. *In vivo* activation of the intracrine vitamin D pathway in innate immune cells and mammary tissue during a bacterial infection. PLoS One 5:e15469. https://doi.org/10.1371/journal.pone.0015469

NRC - National Research Council. 2012. Nutrient requirements of swine. 11th ed. The National Academies Press, Washington, DC.

O'Doherty, J. V.; Gahan, D. A.; O'Shea, C.; Callan, J. J. and Pierce, K. M. 2010. Effects of phytase and 25-hydroxyvitamin D_3 inclusions on the performance, mineral balance and bone parametes of grower-finisher pigs fed low-phosphorus diets. Animal 4:1634-1640. https://doi.org/10.1017/S1751731110000807

Pérez, A. V.; Picotto, G.; Carpentieri, A. R.; Rivoira, M. A.; Peralta López, M. E. and Tolosa de Talamoni, N. G. 2008. Minireview on regulation of intestinal calcium absorption. Emphasis on Molecular Mechanisms of Transcellular Pathway. Digestion 77:22-34. https://doi.org/10.1159/000116623

Pozza, P. C.; Pozza, M. S. S.; Nunes, R. V.; Pasquetti, T. J.; Venturini, I. and Busanello, M. 2010. Desempenho, microbiota intestinal e peso de órgãos de leitões na fase inicial recebendo rações com simbiótico e probiotico. Ciência e Agrotecnologia 34:1327-1334. https://doi.org/10.1590/S1413-70542010000500035

Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Abreu, M. L. T.; Rodrigues, P. B.; Oliveira, R. F.; Barreto, S. L. T. and Brito, C. O. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Departamento de Zootecnia/UFV, Viçosa, MG.

Smith, K. G. and Hunt, J. L. 2004. On the use of spleen mass as a measure of avian immune system strength. Oecologia 138:28-31. https://doi.org/10.1007/s00442-003-1409-y

Schlegel, P.; Guggisberg, D. and Gutzwiller, A. 2017. Tolerance to 1,25 dihydroxyvitamin D_3 glycosides from *Solanum glaucophyllum* by the growing pig. Research in Veterinary Science 112:119-124. https://doi.org/10.1016/j.rvsc.2017.02.013

Schröder, B. and Breves, G. 2006. Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. Animal Health Research Reviews 7:31-41. https://doi.org/10.1017/S1466252307001144

Seedor, J. G.; Quartuccio, H. A. and Thompson, D. D. 1991. The biophosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research 6:339-346. https://doi.org/10.1002/jbmr.5650060405

Stein, H. H.; Adeola, O.; Cromwell, G. L.; Kim, S. W.; Mahan, D. C. and Miller, P. S. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. Journal of Animal Science 89:2139-2144. https://doi.org/10.2527/jas.2010-3522

Teixeira, A. O.; Lopes, D. C.; Vitti, D. M. S. S.; Lopes, J. B.; Gomes, P. C.; Moreira, J. A.; Pena, S. M. and Teixeira, M. P. 2004. Estimativas do fluxo de fósforo entre os compartimentos anatômicos e fisiológicos de suínos alimentados com dietas contendo diferentes fontes de fósforo. Revista Brasileira de Zootecnia 33:1246-1253. https://doi.org/10.1590/S1516-35982004000500017

van Grevenhof, E. M.; Heuven, H. C. M.; van Weeren, P. R. and Bijma, P. 2012. The relationship between growth and osteochondrosis in specific joints in pigs. Livestock Science 143:85-90. https://doi.org/10.1016/j.livsci.2011.09.002

Ytrehus, B.; Carlson, C. S. and Ekman, S. 2007. Etiology and pathogenesis of osteochondrosis. Veterinary Pathology 44:429-448. https://doi.org/10.1354/vp.44-4-429

Wasserman, R. H. 2004. Vitamin D and the dual processes of intestinal calcium absorption. The Journal of Nutrition 134:3137-3139. https://doi.org/10.1093/jn/134.11.3137

White, J. H. 2010. Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: past, present and future. Journal of Steroid Biochemistry and Molecular Biology 121:234-238. https://doi.org/10.1016/j.jsbmb.2010.03.034

Witschi, A. K. M.; Liesegang, A.; Gebert, S.; Weber, G. M. and Wenk, C. 2011. Effect of source and quantity of dietary vitamin D in maternal and creep diets on bone metabolism and growth in piglets. Journal of Animal Science 89:1844-1852. https://doi.org/10.2527/jas.2010-3787

Wongdee, K.; Krishnamra, N. and Charoenphandhu, N. 2012. Endochondral bone growth, bone calcium accretion, and bone mineral density: how are they related? The Journal of Physiological Sciences 62:299-307. https://doi.org/10.1007/s12576-012-0212-0

Zimmerman, D. R.; Koszewski, N. J.; Hoy, D. A.; Goff, J. P. and Horst, R. L. 2015. Targeted delivery of 1,25-dihydoxyvitamin D_3 to colon tissue and identification of a major 1,25-dihydroxivitamin D_3 glycoside from *Solanum glaucophyllun* plant leaves. Journal of Steroid Biochemistry and Molecular Biology 148:318-325. https://doi.org/10.1016/j.jsbmb.2014.10.019

Zhou, H.; Chen, Y.; Zhou, Y.; Lv, G.; Lin, Y.; Feng, B.; Fang, Z.; Che, L.; Li, J.; Xu, S. and Wu, D. 2017. Effects of 25-hydroxycholecalciferol supplementation in maternal diets on milk quality and serum bone status markers of sows and bone quality of piglets. Animal Science Journal 88:476-483. https://doi.org/10.1111/asj.12638