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Early View

Original Research Article

Evaluating Seasonal Serum Vitamin D Levels and Growth Performance in Pigs From Organic Farms

Key words

vitamin D;
serum concentrations;
pigs;
organic farm;
growth performance

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Abstract: Vitamin D is an essential micronutrient in pig production as it plays an important role in many physiological functions. The aim of this study was to determine serum vitamin D concentration and growth performance in pigs reared on organic farms in different seasons. A total of 109 Krškopolje pigs were selected for this study. Pigs were divided into three groups: low altitude outdoor group A (N = 39), high altitude outdoor group B (N = 36) and indoor group C (N = 34). Blood samples and body weights were collected seasonally in 2022 and 2023. Serum vitamin D levels peaked in summer for outdoor groups A (69.3 ±2.6 ng/ml) and B (65.3 ±4.4 ng/ml) and were the lowest in winter (group A 21.5 ±2.2, group B 35.2 ±2.5). Altitude had no significant effect on vitamin D levels except in winter ($p<0.001$). Housing type significantly influenced vitamin D levels in every season between groups B and C, and in spring, summer, and autumn between groups A and C. Pigs in the outdoor group A showed higher average final body weights (151.2 ±7.5 kg in 2022 and 146.8 ±3.8 kg in 2023) than those in group C (132.7 ±11.3 kg and 118.5 ±6.9 kg), though this should be interpreted cautiously given variability in initial weights.

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Introduction

Vitamin D comprises a group of fat-soluble steroid compounds with a similar chemical structure and a variety of physiological functions. It is best known for its role in the homeostasis of calcium in bone formation by increasing calcium absorption from the intestine, stimulating calcium reabsorption in the distal renal tubules, and mobilising calcium stored in bone (1, 2).

The two main forms of vitamin D are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin

D_3). Vitamin D_3 is formed in the skin after exposure to ultraviolet radiation from sunlight (UVB). Vitamin D_3 is then hydroxylated in the liver to 25-hydroxyvitamin D_3 (25(OH)D_3). This metabolite is present in serum, and its concentration is measured to determine vitamin D status in an individual (3). 25(OH)D_3 is further hydroxylated in the kidney to 1, 25-dihydroxyvitamin D_3 or calcitriol, the biologically active form of vitamin D (1, 2). Vitamin D_2 is formed from ergosterol under UVB radiation in fungi and plants (2, 4) and must be ingested with food. Studies have shown differences in the physiological effects of both variants of vitamin D, with vitamin D_3 being more stable, more potent, and more effective in raising serum vitamin D levels than vitamin D_2 (3).

Like many other animals and humans (1, 5, 6), pigs are also able to synthesise vitamin D in their skin. However, as pigs in intensive pork production are mostly raised indoors without access to sunlight, they are dependent on dietary intake of vitamin D. Chronic vitamin D deficiency in pigs may lead to certain metabolic bone diseases (7, 8). Vitamin D deficiency affects bone strength in grower pigs, leading to lameness, rickets with flared growth plates, and bone fractures. Hypocalcemic paresis, tremors, and even sudden death can occur. Swine with clinical rickets generally have serum vitamin D concentrations lower than 5 ng/ml, whereas swine with concentrations above 15 ng/ml appear to be clinically healthy (9). Piglets are most susceptible to hypovitaminosis D, as they are born with the lowest blood concentrations of vitamin D (8).

Vitamin D supplementation positively impacts growth performance, bone formation and immunity (10, 11, 12), meaning it also has an impact on production parameters in the pig industry. In humans, recommendations for adequate serum vitamin D concentrations have already been established (13). In pigs, vitamin D concentrations of 30 ng/ml in the blood are considered minimal standard, but 50 – 80 is required for optimal development (14). Some authors report reference serum vitamin D concentrations for individual categories of pigs (7, 8). To our knowledge, there is only one previous study evaluating serum vitamin D concentrations in organic pig farms (3), which suggested a reference value of 35–99 ng/ml for weaned sows.

In Slovenia, intensive pig farming is in decline (15). However, there are many backyard farms with one or two pigs kept for home consumption, and recently, there has been a surge of organic pig farms. There are 365 organic pig farms registered in the country, keeping around 3000 pigs of all categories (16). Many of these pigs are often reared outdoors or have the possibility of outside access, which gives us the opportunity to study the effects of sun-derived vitamin D in pigs, which is not normally possible in intensive pig production. The aims of this study were to determine the seasonal serum concentrations of vitamin D as well as growth performance of pigs reared on organic farms, and to see whether there is a connection between sun-derived vitamin D and growth performance of pigs.

Materials and methods

Ethics

This study was carried out as part of the ERA-Net CORE Organic Cofound project - RObust Animals in sustainable Mixed Free-range systems project (ROAM-FREE). The study was ethically approved by the Slovenian Ministry of Agriculture, Forestry and Food (U34401-

6/2022/11). The overall objective of the ROAM-FREE project is to investigate how mixed freerange production systems can improve animal welfare, robustness, environmental and economic sustainability and biodiversity in organic pig farming.

Experimental design

A total of 109 grower-to-finisher pigs were selected for this study and were reared in two consecutive years: 2022 and 2023. All animals were Krškopolje pigs, the only autochthonous Slovenian pig breed. Krškopolje pigs are mostly black, medium-sized pigs with a distinctive white belt running across shoulders and forelimbs. The breed was historically reared in modest conditions and is therefore characterised by extreme resilience, great adaptability, adequate reproductive capacity, and quality of meat and fat (17). All this means that Krškopolje pigs are mostly reared in extensive conditions and can be reared outdoors even in winter. All pigs in this study were born on the same organic pig farm in the Littoral-Inner Carniola region in southwestern Slovenia. Pigs were bred to provide organic meat products for consumption. Pigs were divided into groups A, B, and C and distributed to three locations for further rearing.

Figure 1: Outdoor pigs in group A with a visible dugout (Photo: M. Štukelj)

Based on the live weights recorded at the beginning of the experiment, as shown in Tables 5 and 6, and given that the exact ages of the pigs at the time of measurement are unknown, it is not possible to determine their precise age at the start of the experiment. The experiment began on the 21st of April 2022 and ended on the 12th of October 2023, when the last batch of pigs was sampled (Table 1).

Housing and feed

Pigs were divided into three groups: low-altitude outdoor pigs (Group A, N = 39), high-altitude outdoor pigs (Group B, N = 36), and a group of indoor pigs (Group C, N = 34). Pigs in group A were kept in a large, fenced pasture (9000 m^2) with access to direct sunlight. There were no trees in the pasture, but there were two straw-bedded dugouts (13.5 m^2 each) (Figure 1), and a larger straw-bedded, roofed pen (65 m^2) where pigs in group A were housed in winter. Feeders and nipple drinkers were available. Pigs in this group shared the pasture with cattle.

Pigs in group B were reared outdoors on a large grass pasture (12600 m^2) with live wire fencing. Less than half of the pasture area (5400 m^2) was overgrown with trees and bushes. Animals had access to a roofed shelter (40 m^2) and a pigloo. The shelter had straw bedding, one water trough and one feed trough inside. Pigs in this group shared the pasture with sheep (Figure 2).

Pigs in group C (i.e. control group) were housed in two pens (50 m^2 each) within a large barn, without access to sunlight, yet not entirely isolated from daylight (Figure 3). The barn had straw bedding; feeders and nipple drinkers were available for the animals. Natural ventilation was provided year-round, and the building was not heated in winter.

Pigs in groups A and C were kept at an altitude of 550 metres, pigs in group B were kept at an altitude of 820 metres. Approximate altitudes were obtained from publicly available data provided by Surveying and Mapping Authority of the Republic of Slovenia (18). Pigs in groups A and B grazed freely on the pasture, constantly coming in contact with, and possibly ingesting parts of bushes and grass.

Figure 2: High altitude pigs of group B together with sheep. Pigs are visibly sunburned on the white part of their bodies (Photo: M. Štukelj)

Figure 3: Pigs in group C (control group) in the barn (Photo: M. Štukelj)

All groups of pigs were fed the same organically produced feed, which consisted of 60% barley, 30% wheat, and 10% sunflower meal. Feed was grown on site. Pigs in group B were fed twice a day; approximately 35 kilogrammes of feed per group in the morning, and 15 kilogrammes of feed per group in the afternoon (circa 350 kilogrammes every week). Pigs in groups A and C were fed *ad libitum* and consumed approximately 700 kilogrammes of feed each week (or 350 kilogrammes per week each). No additives of any kind were added to the feed, including mineral or vitamin premixes.

Animal welfare, health assessment and treatment

Pigs in this study were reared in accordance with Regulation (EU) 2018/848 of the European Parliament and of the Council of 30th May 2018 on organic production and labelling of organic products (19), as well as the currently valid animal welfare laws and regulations of the state (20). The welfare of the animals and their health status were regularly assessed prior to each sampling in 2022 and 2023.

All pigs in this study were vaccinated against *Erysipelothrix rhusiopathiae* and received a complete treatment against ecto- and endoparasites before the start of the study. The pig herd was certified free of African swine fever, Classical swine fever, Aujeszky's disease, Porcine reproductive and respiratory syndrome (PRRS), *Clostridium perfringens C*, *Brachyspira hyodysenteriae,* and *Salmonella spp*. The herd was certified as free of African swine fever, Classical swine fever, and Aujeszky's disease based on laboratory tests carried out under the annual national Order on monitoring of animal health status, animal disease eradication programmes and vaccinations of animals (21), the rest was tested at the request of the farm's owner. During each visit to the herd, pigs were clinically examined, their welfare was assessed, and weight was calculated. Before each blood sample was taken, the length of the pig's body from the auricular region to the base of the tail and the chest circumference were measured using a tape measure. Weight of each animal in groups A, B and C was calculated using: (*chest circumference (meters)*)^2 x (*body length (meters)*) x *69.3* (22). The weight of the individual animal is given in kilogrammes. Calculations using this method are reported to be accurate to within 3%.

Blood sampling and transport

Blood sampling was performed in four periods, once every season in year 2022 and replicated in 2023, as shown in Table 1**.** We defined individual seasons as follows: Spring (March, April, May), Summer (June, July, August), Autumn (September, October, November), Winter (December, January, February). The exact sampling dates were set depending on the availability of the owner of the farm and the veterinary staff, however, they had to fall within the three-month period of each defined season. The only exception was November 25, 2022, which did not align with the seasonal schedule but was selected as it was the only feasible date for both the veterinary staff and the farmer. Due to its close proximity to December, it was categorized as part of Winter.

Table 1: Dates in which blood was sampled in 2022 and 2023

Season	2022	N	2023	N
Spring	21st April	57	5th May	52
Summer	8th August	57	19th July	52
Autumn	7th October	46	12th October	51
Winter	25th November	36	24th February	52

Pigs were restrained using steel wire snout snare. Blood samples were obtained by puncturing the cranial vena cava with a needle of 15 G – 1.80 x 160 needle (TIK, Slovenia) and a 10/12 ml disposable syringe (KRUUSE, Denmark). Sampled blood was stored in serum tubes (BD-Plymouth, UK) and transported to the laboratory at the ambient temperature of the respective season. Samples in spring and summer were transported in a cool bag. Blood samples were centrifuged at 2500 RPM for 10 minutes and the serum was transferred to separate vials, which were then stored at – 20 °C until analysis. Samples were analysed using Vidas 25 OH Vitamin D TOTAL (Biomerieux, France), an automated quantitative test for the determination of total 25-hydroxyvitamin D (25(OH)D_T) in serum or plasma samples using the ELFA (Enzyme Linked Fluorescent Assay) technique.

Statistical analysis

Measured serum vitamin D levels were arranged by group, sampling date and season and mean values for every season were calculated using Excel (Microsoft, USA).

Data was statistically analysed using statistical software R version 4.3.2. (GiltHub, USA). Gathered data was checked for differences using analysis of variance (ANOVA) and Tukey's HSD test or Welch's t-test, depending on the results of the Bartlett's test for homoscedasticity. We compared data to check for differences in mean values of serum vitamin D between seasons for each group of pigs. We also compared seasonal mean values of vitamin D between groups A and B to determine the effect of different altitudes on serum vitamin D levels and between groups A and C and B and C to see whether different types of housing affect levels of serum vitamin D in pigs. Considering slight imbalance in a few group comparisons, we fitted linear mixed-effects models (LMMs) with Satterthwaite degrees-of-freedom corrections. Initial weight, age (highly correlated with weight), and sex were tested as potential confounders in nested LMMs but none improved model fit. The resulting LMM p-values were marginally larger than those from the standard ANOVA, yet the numerical differences were negligible with our moderate sample sizes. This confirms that, in the absence of meaningful confounders and with all ANOVA assumptions met, the standard factorial ANOVA with Tukey's HSD provides valid and robust inference.

In addition, reference intervals were estimated using the R-package refineR, which implements the recently published, state-of-the-art indirect method (23). It takes routine measurements of diagnostic tests as input and uses sophisticated statistical methods to derive a model describing the distribution of the non-pathological samples. This distribution is then used to derive reference intervals. Mean body weight of pigs between groups A and C in every measurement was checked for differences using Welch's t-test to determine, if the means were statistically significant.

Results

Clinical examinations

Animals showed no apparent signs of disease and were clinically healthy prior to sampling in every season in 2022 and 2023. Pigs in groups A and B were sunburned on the non-pigmented parts of their bodies in the summer of 2022 and 2023 (Figure 2).

Mean values of serum vitamin D

The mean values of total serum vitamin D concentrations in sampled Krškopolje pigs for every season are shown in Table 2 and Figure 4. Statistical significance was found between all seasonal mean values of serum 25(OH)D_T for every pig group.

Table 2: Mean total serum 25(OH)D_T concentrations of different groups of pigs sampled each season

Mean 25(OH)DT	GROUP A (Range)	GROUP B (Range)	GROUP C (Range)	p-values			
				Overall	AB	AC	BC
Spring (ng/ml) Min –max	N = 39[1] 43.5 ± 3.6 (11.3–64.6)	N = 36 34.0 ± 3.3 (11.4–89.0)	N = 34[2] 22.8 ± 2.7 (8.1–51.4)	<0.001	0.13	<0.001	0.023
Summer (ng/ml) Min –max	N = 39 69.3 ± 2.6 (28.1–103.9)	N = 36 65.3 ± 4.4[A] (33.1–124.9)	N = 34[3] 18.8 ± 1.0 (10.6–29.4)	<0.001	0.45	<0.001	<0.001
Autumn (ng/ml) Min –max	N = 37 59.4 ± 2.2 (26.9–79.1)	N = 36 61.4 ± 3.1 (26.3–105.2)	N = 24 28.3 ± 2.5 (12.9–62.8)	<0.001	0.84	<0.001	<0.001
Winter (ng/ml) Min –max	N = 30[4] 21.5 ± 2.2 (8.8–45.7)	N = 36 35.2 ± 2.5 (17.3–70.5)	N = 22 18.6 ± 1.5 (8.9–34.1)	<0.001	<0.001	0.28	<0.001

When evaluating the effect of altitude on total serum 25(OH)D_T concentrations by comparing means of groups A and B, no statistical significance was found in any season other than winter, when the mean value of group B was greater (p<0.001) than that of group A. When evaluating the effect of outdoor/indoor housing of pigs between groups A and C on serum 25(OH)D_T concentrations, means were significantly greater in all seasons in group A, except winter (p=0.28), whereas between groups B and C, means in every season were significantly greater in group B (Table 2).

Reference intervals

Reference intervals were evaluated from combined seasonal values of serum 25(OH)D_T levels together for both groups of outdoor grower-to-finisher pigs (groups A and B) and separately for indoor grower-to-finisher pigs (group C). Reference intervals are shown in Table 3.

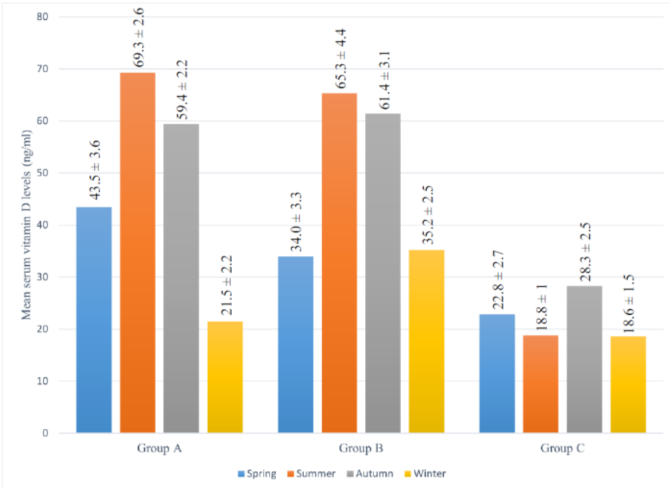

Figure 4: Seasonal serum 25(OH)D_T concentrations for groups A, B, and C

Growth performance of pigs in groups A, B, and C

All groups of pigs were measured at the same time as shown in Table 1. Calculated weight, seasonal growth values, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratios (FCR) are shown in Tables 4 and 5**.** Values were compared only between groups A and C, since they were both fed *ad libitum*.

Table 3: Evaluated reference intervals of combined seasonal values

Reference intervals	Lower limit (2, 5% perc.)	Median (50, 0% perc.)	Upper limit (97, 5% perc.)
Outdoor pigs (ng/ml) Groups A and B N = 269	25.1	55	104
Indoor pigs (ng/ml) Group C N = 112	6.7	16.8	40.6

GROUP A: low altitude outdoor pigs; GROUP B: high altitude outdoor pigs; GROUP C: indoor pigs; N: Combined number of samples from all seasons

Statistical significance between mean body weights in groups A and C (2022) was not found in any measurements other than the third measurement (p=0.015), where the mean body weights were noticeably greater in group A compared to group C (Table 4).

Mean body weights between groups A and C (2023) were significantly greater in group A in every measurement (Table 5).

Table 4: Growth performance of pigs in Group A, B, and C in 2022

Growth performance	GROUP A	GROUP B	GROUP C	p-values			
				Overall	AB	AC	BC
First measurement Weight (kg) Range (kg) 21st April 2022	N = 19 44.7 ± 2.4 22.3–65.9	N = 19 26.9 ± 2.6 10.8–45.6	N = 19 41.2 ± 2.8 18.2–63.9	<0.001	<0.001	0.61	<0.001
Second measurement Weight (kg) Range (kg) ADG (g/day) ADFI (kg/day) FCR 8th August 2022	N = 19 96.1 ± 3.6 50.5–116.8 467.3 2.6 5.6	N = 19 48.5 ± 3.7 23.1–74.5 181.5 2.4 13.3	N = 19[1] 83.9 ± 5.6 40.5–129.0 388.2 2.7 7.5	<0.001	<0.001	0.13	<0.001
Third measurement Weight (kg) Range (kg) ADG (g/day) ADFI (kg/day) FCR 7th October 2022	N = 17 133.5 ± 5.0 87.3–172.7 613.1 2.2 4.7	N = 19 65.4 ± 5.3 30.0–117.8 277.0 1.5 9.3	N = 10 108.1 ± 6.7 56.1–127.9 396.7 4.9 12.4	<0.001	<0.001	0.015	<0.001
Fourth measurement Weight (kg) Range (kg) ADG (g/day) ADFI (kg/day) FCR 25th November 2022	N = 10 151.2 ± 7.5 121.5–199.4 354.0 4.9 13.8	N = 19 80.6 ± 5.8 45.0–127.8 304.0 2.6 8.5	N = 7 132.7 ± 11.3 81.8–168.0 492.0 7.0 14.2	<0.001	<0.001	0.32	<0.001

GROUP A: low altitude outdoor pigs; GROUP B: high altitude outdoor pigs; GROUP C: indoor pigs; [1]: One pig was not measured; N: Number of animals. Number of animals in groups A and C dropped through measurements, due to animals being slaughtered; ADG: Average daily gain between two measurements; ADFI: Average daily feed intake of individual pig; FCR: Average feed conversion ratio of individual pig; **±:** Standard error of the mean (SEM); p-value vas calculated between mean weights of groups A and C

Table 5: Growth performance of pigs in Group A, B, and C in 2023

Growth performance	GROUP A	GROUP B	GROUP C	p-values			
				Overall	AB	AC	BC
First measurement Weight (kg) Range (kg) 24th February 2023	N = 20 34.5 ± 1.4 26.2–45.1	N = 17 18.7 ± 1.3 13.2–33.7	N = 15 18.3 ± 1.3 11.9–26.5	<0.001	<0.001	<0.001	0.97
Second measurement Weight (kg) Range (kg) ADG (g/day) ADFI (kg/day) FCR 5th May 2023	N = 20 59.1 ± 2. 40.5–79.5 346.7 2.5 7.1	N = 17 39.7 ± 2.6 27.3– 68.6 295.8 2.9 11.1	N = 15 45.3 ± 3.1 26.2– 61.2 380.3 3.3 8.6	<0.001	<0.001	0.0012	0.57
Third measurement Weight (kg) Range (kg) ADG (g/day) ADFI (kg/day) FCR 19th July 2023	N = 20 94.2 ± 3.3 70.7–127.9 461.8 2.5 5.3	N = 17 68.0 ± 4.1 46.7–109.1 372.4 2.9 7.8	N = 15 77.3 ± 5.4 41.2–119.7 421.1 3.3 7.8	<0.001	<0.001	0.0097	0.49
Fourth measurement Weight (kg) Range (kg) ADG (g/day) ADFI (kg/day) FCR 12th October 2023	N = 20 146.8 ± 3.8 118.8–184.5 611.6 2.5 4.0	N = 17 107.3 ± 5.0 74.7–149.9 466.0 2.9 6.3	N = 14 118.5 ± 6.9 72.1–155.5 478.5 3.5 7.3	<0.001	<0.001	<0.001	0.56

GROUP A: low altitude outdoor pigs; GROUP B: high altitude outdoor pigs; GROUP C: indoor pigs; N: Number of animals. Number of animals in groups A and C dropped through measurements, due to animals being slaughtered; ADG: Average daily gain between two measurements; ADFI: Average daily feed intake of individual pig; FCR: Average feed conversion ratio of individual pig; **±:** Standard error of the mean (SEM); p-value vas calculated between mean weights of groups A and C

Discussion

This study is the first to investigate total serum vitamin D concentrations in extensively reared Krškopolje pigs on an organic farm and, as far as we are aware, the first study to investigate serum vitamin D in pigs in every season of the year. Studies on serum vitamin D in pigs are lacking, especially in pigs from organic herds. However, there are reported reference values for serum vitamin D concentrations for each individual category of indoor pigs (7, 8). The mean values of 25(OH)D_T serum concentrations for groups A and B peaked in summer with values of 69.3 ± 2.6 ng/ml and 65.3 ± 4.4 ng/ml, respectively. Our results in these two groups correspond with serum 25(OH)D_3 levels of 67 ± 16 ng/ml measured at approximately the same time of year in outdoor sows of the Danish Landrace and Yorkshire cross breeds in organic herds in Denmark in August 2020 (3), as well as serum 25(OH)D_3 levels of 57.2 ± 2.8 ng/ml in outdoor black-skinned sows and 61.1 ± 6.56 ng/ml in outdoor white-skinned grower pigs in the upper Midwest of the United States in June 2011 (8). The aforementioned studies report specifically on serum levels of sun-derived 25(OH)D_3, which means that our results are not directly comparable to theirs, as our study reports total serum vitamin D concentrations (both sun- and feed derived). We do not know what the exact ratio between 25(OH)D_2 and 25(OH)D_3 in our samples is; however, it has been reported in humans that vitamin D_2 is less effective in raising total serum vitamin D (24). In our study, pigs did not receive vitamin D supplements in their feed, meaning that serum 25(OH)D_2 levels are likely to be low. This was also reported in the Danish study, in which serum 25(OH)D_2 levels contributed insignificantly to overall vitamin D status (3).

We noticed a slow decline in serum 25(OH)D_T levels in autumn, and the lowest levels were observed in the winter. The latter was likely due to the fact that Slovenia is geographically located in the Northern Hemisphere at a latitude above 40°, where there is a lack of UVB radiation under which vitamin D can be synthesised (25). Group C had the lowest average serum

25(OH)D_T concentrations; interestingly, however, levels in this group peaked in autumn (28.3 ± 2.5 ng/ml). Another factor plays an important role in the synthesis of sun-derived vitamin D in pigs studied: the pigmentation of their skin. Krškopolje pigs are predominantly black-skinned (Figures 1 to 3), i.e. they have larger amounts of melanin. Studies in humans have shown that increased melanin levels effectively reduce vitamin D synthesis in the skin, which in turn lowers serum vitamin D concentrations (26, 27). This would mean that the observed serum vitamin D concentrations in Krškopolje pigs are lower than would be expected if we included whiteskinned pig breeds (e.g. Landrace) under the same conditions. The number of pig sera sampled gradually decreased in each subsequent season. This was due to the fact that the pigs were taken to slaughter. In the spring of

2023, 19 blood samples from group A yielded results below 8.1 ng/ml, which is beneath the minimum detection threshold of the Vidas 25 OH Vitamin D TOTAL assay. As the exact 25(OH)D_T concentrations could not be determined, these samples were excluded from the study. Excluded samples showed moderate to severe hemolysis.

Serum 25(OH)D_T concentrations of Krškopolje pigs show a high variation within groups A and B (Table 2), with the highest ranges observed in summer (28.1–103.9 ng/ml and 33.1–124.9 ng/ml), which is comparable to the 32–134 ng/ml reported in the sera of newly weaned sows in Danish organic farms (3). Pigs in the outdoor groups A and B were housed in fenced areas containing trees, bushes, pens, and a pigloo, allowing them constant access to shelter from direct sunlight. However, we had no data on individual sunlight exposure, solar intensity, day length, or weather conditions (e.g., cloud cover or storms). During summer and autumn, many of the tested pigs exhibited mild to moderate sunburns on non-pigmented skin areas (Figure 2), suggesting prolonged exposure to direct sunlight. These environmental and behavioural factors may account for the substantial variation in serum 25(OH)D_T concentrations among individual pigs.

Groups of outdoor pigs were reared at different altitudes, providing an opportunity to investigate whether altitude affects serum 25(OH)D_T concentrations. In humans, higher altitude has been reported to be associated with increased vitamin D production (25). Pigs in group A were reared at an altitude of 550 meters, while those in group B were raised at 820 meters. A statistically significant difference was observed only when comparing mean serum 25(OH)D_T concentrations between groups A and B in winter ($p<0.001$), with higher values in group B. No significant differences were found between groups during the other seasons. Therefore, we conclude that altitude had no effect on serum 25(OH)D_T concentrations in spring, summer, and autumn—likely because the difference in altitude was not substantial enough to have a meaningful impact. In winter, however, the mean values were higher in group B, which may be attributed to more effective UVB exposure at the higher altitude, resulting in a slower decline in vitamin D levels during this season. Pigs in group B only had a pigloo and a smaller shelter (40 m^2) available, while the pigs in group A had a larger shelter available (65 m^2), which means that the pigs in group B most likely spent more time in the sun, even in winter. To our knowledge, no similar studies have been conducted in pigs. However, one study examining the influence of altitude on vitamin D status in lactating sheep and goats (28) also reported no significant effect. We compared the effect of housing on serum 25(OH)D_T concentrations between outdoor and indoor pigs. Mean values across all seasons were significantly higher in group A compared to group C, except in winter, where no significant difference was observed ($p=0.28$). In contrast, mean serum concentrations in group B were significantly higher than those in group C across all seasons. These results confirm that pigs housed outdoors have higher serum vitamin D levels than those kept indoors. This corresponds with the results from the Midwest of the United States, where authors also reported significantly greater serum vitamin D levels in outdoor pigs compared to pigs raised in confinement (8). One study did a similar comparison of serum vitamin D levels in White Landrace-Duroc and Yorkshire-DurocLandrace crossbred pigs by exposing them to sunlight in summer and autumn (28). They similarly reported higher post-exposure levels compared to pigs housed indoors, but interestingly, serum levels in that study were higher in pigs exposed in autumn than in summer.

Krškopolje pigs in our study were clinically examined prior to every sampling, and none of them showed any clinical signs of hypovitaminosis. Mean seasonal values of all groups are within or higher than previously reported reference levels of serum vitamin D for confined grower pigs (10–30 ng/ml) (7, 8). In this study, values from groups A and B from all four seasons in 2022 and 2023 were combined; the same was done with values from group C. Reference intervals were subsequently evaluated for outdoor pigs (groups A and B) and indoor pigs, respectively (Table 4). As all animals were clinically healthy, these results could serve as reference levels for total serum vitamin D for outdoor grower-to-finisher pigs (25.1–104 ng/ml) and indoor grower-to-finisher pigs (6.7–40.6 ng/ml) reared on organic farms.

Pigs were examined by a veterinarian prior to every blood sampling and were clinically healthy throughout the entire study. All pigs were measured, and subsequently their mean seasonal body weight, ADG, ADFI, and FCR were calculated separately for 2022 and 2023 (Tables 4 and 5). Pigs in groups A and C were reared at the same location and received the same diet, with housing conditions being the only planned difference—group A was kept outdoors, while group C was housed indoors. Although group A pigs generally showed higher average body weights than group C pigs across seasons in both 2022 and 2023, these differences should be interpreted with caution due to the considerable variability in initial body weights, the unknown and likely differing ages of the animals, and the unknown sex distribution within groups. Pigs in group A reached the highest average body weight among all groups in the final (fourth) measurement.

In 2022, the greatest ADG in group A occurred in the third measurement, whereas the greatest ADG was observed in the fourth measurement. A statistically significant difference between the mean body weights of groups A and C was detected only in the third measurement ($p=0.015$), where pigs in group A had significantly higher average body weights. In 2023, a different growth pattern emerged: ADG generally increased across the measurement periods, with the most substantial growth occurring between the third and fourth measurements.

Statistically significant differences between the groups were found in all measurement periods in 2023, with pigs in group A consistently exhibiting higher average body weights than those in group C. Nonetheless, these outcomes should be viewed in the context of the study's limitations, including the lack of data on the animals' exact ages and sexes, as well as the initial variability in body weight.

In 2023, FCR corresponded with ADG in the individual measurements; the FCR values were lowest when ADG were highest. Lower FCR values indicate better conversion of feed into body tissue (30). This was not the case in 2022. This could be explained by animals being sent to slaughter, meaning less animals were able to consume feed. Normally, pigs undergo rapid growth in the early stages, until reaching a maximum. At higher weights, growth rate declines as pigs are reaching maturity (S-shaped growth curve) (31). Different trends in growth in 2022 and 2023 could be explained by the fact that the individual measurements were not carried out at the exact same time in both years. In group B, both seasonal body weights were noticeably lower than in groups A and C. This was likely due to the fact, that pigs in group B were fed twice a day, whereas pigs in groups A and C were fed *ad libitum*. This would have allowed pigs in groups A and C prolonged access to feed, resulting in greater body weights and weight gains compared to pigs in group B. In all measurements, there was a rather large range in body weight within every pig group. The reason for this was likely that individual animals spent more time feeding than others; there could also have been some pathogens present in the herd (e.g., porcine circovirus 2), which could decrease growth performance, or it could be related to physiological differences between individual animals. At the start of the study, pigs were not of equal body weight, and those with smaller initial weights normally did not grow as fast as their peers. Pigs in groups A and B were reared outdoors and were thus able to express their natural behaviour. Pigs under natural conditions spend up to 75% of their daily activity engaged in rooting and foraging (32), which means they likely spent more time searching for food and could therefore ingest extra food in addition to the feed they were given daily. Expression of natural behaviour could be one of the reasons that pigs in group A reached larger body weights than their indoor peers. Some studies have shown that adding vitamin D_3 to the feed impacts production parameters by increasing body weight and weight gain in pigs (33) and broilers (34). Pigs in our study received no dietary supplement of vitamin D; however, pigs in group A also had higher serum vitamin D concentrations than group C. Even though pigs in group A showed better production parameters (e.g., reaching larger weights and having lower FCR), this study cannot directly confirm that pigs from group A exhibited better production parameters due to higher serum vitamin D levels, but it is a question that should definitely be investigated further.

Our study is not the first to evaluate growth parameters in Krškopolje pigs. In 2017, Tomažin et al. (35) compared Krškopolje pigs reared under extensive and intensive conditions. Contrary to our findings, they reported that Krškopolje pigs reared under intensive conditions exhibited significantly higher growth rates and reached heavier slaughter weight (120 kg) compared to pigs reared under extensive conditions (88 kg). It is worth noting, however, that intensively reared pigs in that study were fed a complete feed mixture, whereas extensively reared pigs were only fed traditional cooked feed with some ground grains. Another study from 2024, Škrlep et al. (36) compared Krškopolje pigs with modern hybrid breeds. Krškopolje pigs showed slower growth rates compared to modern breeds, but were, interestingly, less affected by reductions of dietary protein levels, indicating lower protein requirement and better adaptability.

Throughout the study, the Krškopolje pigs were exposed to challenging environmental conditions, as well as the rugged Karst terrain, and yet they showed excellent health status, good serum vitamin D concentrations, adequate feed conversion, and good growth. For this reason, the Krškopolje pigs can be described as "robust". The term "robustness" was defined by Knap (37) as the ability to combine high production potential with resilience to stress factors, allowing for expression of high production potential under a variety of environmental conditions. Krškopolje pigs owe their robustness to the modest environmental conditions in which they were historically reared. Of course, their production parameters are not comparable to those of intensive pork production, but their resilience and robustness make them well-suited for organic pig farming.

Conclusion

We reported total serum vitamin D concentrations in 109 grower-to-finisher pigs reared outdoors and indoors in organic pig farms. Sampling was performed once every season in 2022 and 2023. Serum vitamin D concentrations in outdoor pigs were highest in summer and lowest in winter. Values in both outdoor groups showed high individual variation. When evaluating the effects of altitude on serum vitamin D concentrations, we concluded that altitude did not significantly affect serum vitamin D concentrations in either group of outdoor pigs in any season, except winter. We concluded that serum vitamin D levels were significantly higher in outdoor pigs than in indoor pigs. Reference intervals calculated in this study could serve as reference levels of total serum vitamin D for outdoor and indoor organic grower-to-finisher pigs. Outdoor pigs in group A tended to achieve higher final body weights compared to indoor pigs in group C; however, this apparent difference in growth performance should be interpreted with caution due to considerable variability in initial body weights, unknown ages, and unrecorded sex distribution among the animals. These findings highlight the benefits of outdoor rearing for vitamin D status and growth performance in pigs.

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