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Addition of calcitic seaweed in the diet of sows positively affects the number of live-born piglets and milk parameters

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ABSTRACT - This study was conducted to assess the effects of maternal dietary calcitic seaweed (CSW) on performance and blood metabolites of sows, and on performance, blood metabolites, intestinal microbiota, and parameters of gastrointestinal tract and bone of litters. On d 21 (post-insemination), non-pregnant sows were removed from the trial, remaining 19 sows in control group (without CSW) and 16 sows receiving CSW. Then, a total of 35 sows were allocated in a randomized block design with two treatments: control diet with calcitic limestone plus dicalcium phosphate (CTL) or CTL plus 0.4% CSW. In gestation, sows were fed twice a day (07:00 and 15:00 h) to reach an intake of 2.5 kg animal^{-1} day^{-1} divided into two equal meals. On parturition day, sows were offered only 0.5 kg feed animal^{-1}. Throughout lactation, sows were fed three times a day (≅7 kg animal^{-1} day^{-1}). All diets were provided as mash. Results suggested that sows fed CTL had litters with lower body weight at birth compared with those fed CSW. Sows fed CSW had 14.28% more live-born piglets and lower stillborns. Piglets from sows fed CSW showed greater calcium concentration on d 14 after birth than those from sows fed CTL. Sows fed CSW showed better milk chemical composition and an increase of 27.16% in milk production compared with those fed CTL. Piglets from sows fed CSW had an increase in cecum content in the Enterobacteriaceae count. This study showed that adding 0.4% CSW in the diet of pregnant and lactating sows as an organic calcium source positively influences the number of live-born piglets and the percentage of stillborns. In addition, milk composition and production are also improved without affecting piglets' biological response.

Keywords: calcium sources, *Lithothamnium calcareum*, litter performance, milk, sows

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1. Introduction

Special attention should be given to the nutritional requirements of modern hyperprolific sows (Silva et al., 2018). Thus, performance improvements have raised concerns to adjust dietary minerals

such as calcium (Barrilli et al., 2017). However, calcium content and bioavailability may change among ingredients (González-Vega and Stein, 2016). Furthermore, calcium metabolism can be affected by variation in calcium concentration and bioavailability, intrinsic animal traits (González-Vega et al., 2014), interaction with other nutrients, and additives (González-Vega et al., 2015).

On this note, calcium plays a key role in the nutrition of sows, as well as in their offspring (Tan et al., 2016). Fetal requirements for minerals are higher at the end of pregnancy, and mobilization of body reserves before lactation is needed when dietary concentration is insufficient (Gaillard et al., 2020). Proper diet balancing for calcium, according to female category and litter size (Gaillard et al., 2019), prevents bone demineralization due to fetal growth and milk production (Tokach et al., 2019).

Primary sources of calcium-containing dietary supplements for pigs are inorganic (Barrilli et al., 2017). Even though calcium carbonate is the most commonly used source, *Lithothamnium calcareum* can be used as an organic source (Santos et al., 2021). *Lithothamnium calcareum* is a fossil belonging to the group of red seaweed from Coralineacea family (Almeida et al., 2012). It has calcitic aspect due to calcium carbonate absorption and is not a source of protein, vitamin, carbohydrates, nor fat (Melo and Moura, 2009).

Studies have reported the effect of calcium feeding time on sow performance (Gao et al., 2019), as well as the importance of using more bioavailable sources (Barrilli et al., 2017). The effects of maternal ingestion of organic sources on fetal growth and litter bone development are not clearly defined for pigs (Ji et al., 2017). When given to piglets, *L. calcareum* can affect mineral utilization, bone and stomach traits (Schlegel and Gutzwiller, 2017), digesta pH (González-Vega et al., 2014), blood parameters (Santos et al., 2021), and intestinal health (Leonard et al., 2011; Heim et al., 2014a,b; Heim et al., 2015).

Here, the hypothesis of this article was that supplementation with calcitic seaweed in diets would promote performance improvements in sows, consequently, positively influencing their litters. Therefore, the present study aimed to assess the effects of maternal dietary calcitic seaweed on performance and blood metabolites of sows and on performance, blood metabolites, intestinal microbiota, and parameters of gastrointestinal tract and bone of litters.

2. Material and Methods

This study was carried out at a commercial piglet production farm located in Marechal Cândido Rondon, Paraná, Brazil (24°30'00.01" S and 54°04'22.79" W). Research on animals was conducted (protocol no. 31/2019) according to the institutional committee on animal use (protocol no. 34/2020).

2.1. Experimental design, animals, housing, and treatments

At the beginning of the experimental period, 52 sows were randomly selected (DB-DanBred swine genetics) to be used in the study. On d 21 (post-insemination), non-pregnant sows were removed from the trial, remaining 19 sows in control group (calcitic seaweed free) and 16 sows receiving calcitic seaweed (CSW). Batch over time (round) was considered as a block and sow in each pen was considered as an experimental unit. Sows were classified by parity in five groups: P1 (12 sows), P2 (14 sows), P3 (five sows), P4 (two sows), and P5 (two sows). The average farrowing order of sows was 2.10 and 2.06 to the control and CSW-fed group, respectively.

After conception, animals were weighed, identified with an ear tag, and housed for 105 days in a masonry room with ceramic roof, partially slatted concrete floor, exhaust fans, and sprinklers. The facility had central aisle with pens (2.1 m^2) equipped with gutter feeders and nipple drinkers on both sides. On d 106 of gestation, sows were moved into a farrowing room equipped with individual masonry pens, slatted plastic flooring, and side bars to avoid crushing of piglets. Pens had masonry skimmers, individual feeders, and nipple drinkers for piglets and sows. Animals remained in this facility until weaning on d 27 after birth.

The diets were formulated to meet the nutritional requirements of breeding pigs according to their production phase (Rostagno et al., 2017). Treatments consisted of two diets offered to sows throughout gestation and lactation phase: control diet with calcitic limestone plus dicalcium phosphate (CTL) or CTL plus 0.4% CSW (Table 1). The experimental dose of CSW (34% total calcium) was chosen based on pilot studies conducted by the company and was added as top-dressing.

Sows were fed twice a day (07:00 and 15:00 h) to reach an intake of 2.5 kg animal^{-1} day^{-1} divided into two equal meals. Daily feed was previously weighed and stored in identified plastic bags. On parturition day, sows were offered only 0.5 kg feed animal^{-1}. Throughout lactation, sows were fed three times a day (≅ 7 kg animal^{-1} day^{-1}). All diets were provided as mash.

Table 1 - Centesimal and chemical composition of experimental diets for sows in gestation and lactation phases (as-fed basis)

Item	Treatment			
	Control		Calcitic seaweed[1]	
	Experimental phase			
	Gestation	Lactation	Gestation	Lactation
Ground corn, 7.59% CP	77.00	63.00	77.00	63.00
Soybean meal, 46.07% CP	20.00	30.00	20.00	30.00
Whey powder, 12.87% CP	-	4.00	-	4.00
Nucleus[2]	3.00	3.00	3.00	3.00
Calculated composition				
Crude protein (CP, %)	15.51	19.11	15.51	19.11
Lactose (%)	-	3.13	-	3.13
Metabolizable energy (MJ/kg)	13.50	13.39	13.50	13.39
Standardized digestible lysine (%)	0.680	0.946	0.680	0.946
Standardized digestible methionine + cysteine (%)	0.453	0.541	0.453	0.541
Standardized digestible tryptophan (%)	0.162	0.217	0.162	0.217
Standardized digestible threonine (%)	0.489	0.640	0.489	0.640
Crude fiber (%)	3.18	3.34	3.18	3.34
Total calcium (%)[3]	0.740	0.800	0.870	0.930
STTD phosphorus (%)[4]	0.061	0.103	0.061	0.103
Total phosphorus (%)[3]	0.496	0.528	0.496	0.528

[1] Addition of calcitic seaweed (34% total calcium) as top-dressing at a proportion of 0.4%.
[2] Nucleus composition of experimental diets provided to sows: crude protein, 150.00 g/kg; total calcium, 197.00 to 240.00 g/kg; total phosphorus, 70.00 g/kg; total sodium, 0.051 g/kg; lysine, 13.00 g/kg; methionine, 2.00 g/kg; phytase, 1,000 units/kg; biotin, 8.00 mg/kg; nicotinic acid, 530.00 mg/kg; pantothenic acid, 375.00 mg/kg; folic acid, 38.00 mg/kg; choline, 5,000 mg/kg; iodine, 24.00 mg/kg; selenium, 10.00 mg/kg; iron, 1,800 mg/kg; copper 4,000 mg/kg; zinc, 3,300 mg/kg; manganese, 1,200 mg/kg; cobalt, 21.00 mg/kg; chromium, 1.00 mg/kg; vitamin K3, 68.00 mg/kg; vitamin B1, 33.00 mg/kg; vitamin B2, 105.00 mg/kg; vitamin B6, 33.00 mg/kg; vitamin B12, 530.00 mg/kg; vitamin A, 215,000 IU/kg; vitamin D3, 72,000 IU/kg; vitamin E, 900.00 IU/kg. Basic composition of the nucleus: meat and bone meal, sugar, calcitic limestone, dicalcium phosphate, sodium chloride (common salt), L-lysine, vitamin A, vitamin D3, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin H, nicotinic acid, pantothenic acid, folic acid, choline chloride, organic chromium, sodium selenite, calcium iodate, iron sulfate, copper sulfate, zinc oxide, manganese sulfate, cobalt sulfate, phytase, antioxidant additive, and vehicle q.s.p.
[3] Total phosphorus and calcium analyzed.
[4] Standardized total tract digestible phosphorus, without considering the available phosphorus from the nucleus.

2.2. Performance testing

Total feed intake (TFI), initial body weight (IBW), final body weight (FBW), average daily gain (ADG), feed conversion ratio (FCR), total numbers of piglets born, born alive, cross-fostering, and stillborn (%), litter and piglet body weight at birth (kg), litter body weight, and average daily gain at weaning (kg) were evaluated. Leftovers and waste were weighed to calculate the feed intake of each sow per phase. Sows were weighed at the beginning and the end of each phase and body weight loss during lactation was calculated.

Body condition score was estimated at the end of lactation using a caliper on the last rib of each sow. After attaching the equipment to the sow skin (without pressing), the marked score was measured. Sows were classified as T = thin, I = ideal, and F = fat as previously described by Knauer and Baitinger (2015). Afterward, the percentage of sows with ideal body condition was calculated.

2.3. Blood sampling

Blood samples (≅ 10 mL) were collected from anterior cranial vena cava at 08:00 h, on days 60 and 90 of gestation, on d 12 of lactation, and on days 14 and 27 after birth in piglets (two animals per pen). Samples were withdrawn using 1.2×40 and 0.7×30 mm needles for sows and piglets, respectively. After sampling, each blood sample was transferred to two different sterile vacuum glasses (one containing heparin and the other containing potassium fluoride). All samples were transported to the lab in a thermal box (4 °C). Plasma was isolated from blood by centrifugation (Centrilab centrifuge, model 80-2B) at 3,000 *g* for 10 min. Plasma samples (duplicates) were stored in microtubes (1.5 mL) at −20 °C until urea (enzymatic-colorimetric method, Cat. 427), glucose (enzymatic-colorimetric Trinder method, Cat. 434), and calcium (O-cresolphthalein-colorimetric method, Cat. 448) analyses. The analyses were performed using commercial kits (Gold Analisa) and an absorption spectrophotometry device (model SP-22, Biospectro brand, São Paulo, SP, Brazil).

2.4. Management of piglets in farrowing room

Parturitions were monitored to adequate management of piglets and evaluation of postpartum variables. After birth, piglets were dried off (Pig Sec, Costavet®), and each litter was individually weighed using a digital scale (model UL-50, Digi-tron brand, Curitiba, PR, Brazil). Afterward, piglets were placed next to the sow's underline for colostrum intake. On d 3, piglets were dewormed (Ripercol, Zoetis®), injected with iron-dextran (Ferrodex, Fabiani®), tail-docked, and identified in the ears. On d 7, mash feed was provided to piglets in collective feeders located on the sides of pens.

2.5. Analysis of milk production and composition

Milk samples (≅10 mL) were collected on d 14 of lactation. Sows were milked manually, and samples (duplicates pooled from functional teats) were stored inside sterile containers. Immediately after sampling, density (kg per m^3), total solids (%), fat (%), crude protein (%), lactose (%), and ash (%) were determined in milk samples using a milk analyzer (Milkoscope Expert Automatic model, brand Tex Tech, Cataguases, MG, Brazil).

Daily milk production was calculated based on litter growth rate and the number of piglets weaned during lactation, according to the equation described by Noblet and Etienne (1989).

2.6. Piglet slaughter and sampling

At the end of lactation, piglets of each treatment (n = 6) were slaughtered after a six-hour fasting period, following a humane slaughter method (electronarcosis with 240 volt for three seconds followed by exsanguination). Data and samples were collected for analyses of pH of digestive tract contents, intestinal microbiota, intestinal epithelial morphometry, and bone parameters (third metacarpal). Animals with body weight closest to the group average were chosen to be slaughtered.

2.7. pH of digestive tract contents, morphometry, and intestinal microbiology

After slaughter, pH of digestive tract contents was evaluated using a digital pH meter (model TEC-2 mp, brand TECNAL, Piracicaba, SP, Brazil).

Jejunum samples (3 cm) were collected (150 cm cranial to ileocecal junction) to measure villi height (VH), crypt depth (CD), and VH:CD ratio (Guo et al., 2001). Fragments collected were washed with

saline (0.9% sodium chloride) and stored in sterile plastic containers with 10% buffered formalin solution. Samples were sent to a commercial lab (Cascavel, PR, Brazil) where they were processed in paraffin. Slides were prepared via hematoxylin and eosin technique as previously reported by Kraieski et al. (2017). Histological analyses were performed using an optical microscope (CX31RTSF, Olympus brand, Tokyo, Japan) and a computer system (ToupView x86). The height of 10 villi was measured and respective crypts were analyzed to calculate the average per animal.

Jejunum, ileum, cecum, and colon content samples were used for enterobacteria (EMB levine agar, Kasvi) and lactic acid bacteria (LAB; MRS agar, Acumedia) counts. Samples (≅20 mL per bottle) were stored in sterile plastic bottles in a thermal box (4 °C) and transported to the laboratory. Subsequently, 1 g of digestive tract contents sample was transferred to sterile tubes and then subjected to serial dilution in saline (0.9%). The 10^{-1} dilution (1 g of sample with 9 mL of saline) was vortexed (model AP 56; Phoenix brand, Araraquara, SP, Brazil) for 30 s. The other dilutions (up to 10^{-6}) were vortexed (model AP 56; Phoenix brand, Araraquara, SP, Brazil) for 10 s. An aliquot (100 µL) of each dilution was seeded by surface spreading with the aid of a Drigalski loop in the appropriate culture media (Weedman et al., 2011). To detect populations of enterobacteria, Petri dishes containing the inoculum were incubated in aerobic ovens at 37 °C for 24 h. To detect LAB populations, Petri dishes containing the inoculum were incubated in anaerobic ovens at 37 °C for 48 h. Afterward, microbiological count data were log-transformed.

2.8. Bone strength and densitometry

After slaughter, fore and hind legs of all animals were collected and placed in identified bags. Bones were manually cleaned and frozen at −5 °C. The third metacarpals of each animal were sent for bone density analysis using the Hologic Discovery Wi® software in small animal mode. Bone strength was performed in a Universal Mechanical Testing Machine (DL 10.000, brand EMIC, with cell 200 kgf EMIC load). Data, expressed as Newtons, were collected directly by a computer coupled to the machine and then transformed into kilogram-force per square centimeter (kgf per cm^2).

2.9. Statistical analysis

Statistical analyzes were performed using the SAS (Statistical Analysis System, University Edition). Residual error was evaluated for outliers via Student's test. If studentized residuals exceeded 3, the sample was removed from statistical analysis. Data were analyzed for the normality of residues via the Shapiro-Wilk test.

For gestation and lactation data, the statistical model included the fixed effect of treatment and the random effect of block. Sow parity and initial body weight were used as covariates. For all other results, the aforementioned model was used without including the covariates. Treatment effects were verified via ANOVA or ANCOVA using the F test. The statistical model used was:

$$Y_{ijk} = \mu + T_i + b_j + \varepsilon_{ijk},$$

in which Y_{ijk} = average observation of the dependent variable in each plot, measured in the i-th treatment class, at the j-th block, and in the k-th replication; μ = effect of the overall average; T_i = fixed effect of treatment classes, for i = 1 and 2; b_j = block effect, for j = 1 and 2; and ε_{ijk} = random error of the plot associated with i-th level, j-th block, and k-th replication.

For live piglets per sow, percentage of stillborns, and ideal body condition, a generalized linear model (GLM) was fitted. Treatment was considered as fixed effect and block as random effect. The GLM used was represented by the systematic portion:

$$\eta = \mu + T_i + b_j,$$

wherein μ was the effect associated with the overall average; T_i was the effect associated with i-th treatment class, for i = 1 and 2; and b_j was the effect associated with j-th block, for j = 1 and 2. The

Akaike information criteria was used to test the model fitting. Treatment effects were tested via type III analysis. Differences were declared significant when P≤0.05, and data are presented as means and their standard error.

3. Results

3.1. Performance testing

Sows fed control diet had litters with lower (P = 0.024) body weight compared with those sows fed CSW. Differences were observed for the number of live born piglets (P = 0.025), percentage of stillborns (P = 0.037), and number of cross-fostered piglets (P = 0.026). Sows fed CSW showed greater live born piglets and lower number of stillborns than those fed control diet (Table 2).

Table 2 - Productive performance of gestating and lactating sows fed diets containing calcitic seaweed

Parameter	Treatment[1]		SEM	P-value[2]		
	Control	Calcitic seaweed		Diet	Cov[3]	Cov[4]
			Gestation phase			
TFI (kg)	220.9	221.4	1.611	-	-	-
IBW (kg)	173.16	189.44	4.000	-	-	-
FBW (kg)	222.89	238.50	4.729	0.536	0.333	0.000
DBWG (kg)	0.48	0.48	0.022	0.875	0.966	0.157
FCR (kg:kg)	4.46	4.32	0.222	0.291	0.968	0.008
Total born piglets (n)	13.37	13.62	0.624	0.886	0.719	0.533
Litter weight at birth (kg)	18.33b	19.89a	0.581	0.024	0.354	0.305
Piglet weight at birth (kg)	1.37	1.43	0.039	0.131	0.311	0.081
			Lactation phase			
TFI (kg)	153.02	154.57	1.547	0.905	0.109	0.060
IBW (kg)	222.89	238.50	4.729	-	-	-
FBW (kg)	198.28	203.09	2.863	0.105	0.001	<0.000
Live-born piglets (n)	11.62B	13.28A	0.624	0.025	-	-
Stillbirths (%)	9.94A	6.74B	0.246	0.037	-	-
Cross-fostering piglets (n)	11.00b	12.19a	0.293	0.026	0.173	0.030
IBW of litter (kg)	17.87	20.23	0.798	0.098	0.106	0.016
Piglets at weaning (n)	10.05	10.69	0.345	0.294	0.098	0.373
FBW of litter (kg)	66.38	68.93	2.755	0.688	0.171	0.072
DBWG of litter (kg)	1.83	1.84	0.092	0.953	0.295	0.223
DBWL of sow (kg)	0.34	0.38	0.035	0.440	0.011	0.000
Optimal body condition (%)	40.00	56.00	0.735	0.067	-	-

TFI - total feed intake; IBW - initial body weight; FBW - final body weight; DBWG - daily body weight gain; FCR - feed conversion ratio; DBWL - daily body weight loss; SEM - standard error of the mean.
A,B - Observed proportions followed by different uppercase letters in the row differ by type III analysis at P≤0.05.
a,b - Average values followed by different lowercase letters in the row differ according to the F test at P≤0.05.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.
[3] Cov: effect of the covariate sow farrowing order.
[4] Cov: effect of the covariate initial body weight of sow.

3.2. Blood metabolites

There were no differences (P>0.05) between treatments for blood metabolites in pregnant and lactating sows (Tables 3 and 4). However, piglets from females fed CSW showed greater (P = 0.022) calcium concentration on d 14 after birth (Table 4).

Table 3 - Average glucose, urea, and calcium concentrations (mg/dL) of gestating sows fed diets containing calcitic seaweed

Parameter	Treatment[1]		SEM	P-value[2]
	Control	Calcitic seaweed		
	Gestation phase - 60 days of experimentation			
Glucose	91.19	101.28	5.112	0.315
Urea	30.88	31.85	1.300	0.669
Calcium	10.09	10.13	0.175	0.966
	Gestation phase - 90 days of experimentation			
Glucose	76.51	71.60	2.422	0.330
Urea	32.49	31.20	0.921	0.403
Calcium	9.48	9.73	0.207	0.486

SEM - standard error of the mean.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.

Table 4 - Average glucose, urea, and calcium concentrations (mg/dL) of lactating sows and suckling piglets fed diets containing calcitic seaweed

Parameter	Treatment[1]		SEM	P-value[2]
	Control	Calcitic seaweed		
	Lactation phase			
Glucose	83.37	88.54	2.486	0.292
Urea	46.01	42.06	2.098	0.157
Calcium	9.62	9.69	0.140	0.600
	Suckling piglets - day 14			
Glucose	157.32	152.42	4.663	0.704
Urea	24.39	21.44	0.802	0.068
Calcium	10.40b	10.93a	0.113	0.022
	Piglets at weaning - day 27			
Glucose	132.57	130.11	3.043	0.576
Urea	22.21	19.15	0.911	0.099
Calcium	9.79	10.25	0.132	0.079

SEM - standard error of the mean.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.
a,b - Average values followed by different lowercase letters in the row differ according to the F test at P≤0.05.

3.3. Milk production and composition

Milk composition was analyzed on d 14 of lactation. Sows fed CSW produced 27.16% more (P = 0.039) milk than those fed control diet (Table 5). Additionally, there was a greater density (P = 0.029), defatted total solids (P = 0.025), crude protein (P = 0.027), lactose (P = 0.026), and ash (P = 0.026) in milk of sows fed CSW.

3.4. Digestive tract contents pH, morphometry, and intestinal microbiota

There was no treatment effect (P>0.05) on digestive tract contents pH and intestinal morphometry of piglets (Table 6). However, piglets from CSW-fed sows showed greater (P = 0.032) Enterobacteriaceae counts in cecal content. No differences were observed (P>0.05) for LAB count in gastrointestinal tract portions (Table 7).

Table 5 - Chemical composition and milk production of lactating sows fed diets containing calcitic seaweed

Parameter	Treatment[1]		SEM	P-value[2]
	Control	Calcitic seaweed		
Density (kg/m^3)	35.19b	36.76a	0.361	0.029
Total solids (%)	17.88	18.19	0.193	0.515
Total defatted solids (%)	10.84b	11.24a	0.088	0.025
Fat (%)	7.04	6.95	0.171	0.666
Crude protein (%)	3.98b	4.13a	0.032	0.027
Lactose (%)	5.95b	6.17a	0.048	0.026
Ash (%)	0.89b	0.93a	0.007	0.026
Milk production (kg/day)	9.24b	11.75a	0.597	0.039

SEM - standard error of the mean.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.
a,b - Average values followed by different lowercase letters in the row differ according to the F test at P≤0.05.

Table 6 - Additional dietary effect of calcitic seaweed in sow diets on pH of the digestive tract contents and intestinal morphometry of piglets

Parameter	Treatment[1]		SEM	P-value[2]
	Control	Calcitic seaweed		
pH of the digestive tract contents				
Stomach	3.51	3.22	0.149	0.238
Jejunum	6.38	6.22	0.087	0.389
Ileum	6.15	6.47	0.138	0.297
Cecum	6.00	6.15	0.067	0.974
Colon	6.60	6.91	0.110	0.252
Jejunum				
Villus height (VH; µm)	555.30	679.40	0.049	0.245
Crypt depth (CD; µm)	290.00	341.33	0.032	0.479
VH:CD ratio	2.25	2.45	0.294	0.722
Ileum				
VH (µm)	387.17	358.83	0.029	0.668
CD (µm)	154.17	148.17	0.013	0.840
VH:CD ratio	2.74	2.81	0.267	0.909

SEM - standard error of the mean.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.

Table 7 - Additional dietary effect of calcitic seaweed in sow diets on microbial population of Enterobacteriaceae and lactic acid bacteria in piglets

Parameter	Treatment[1]		SEM	P-value[2]
	Control	Calcitic seaweed		
Enterobacteriaceae count (Log_{10} colony-forming units/g)				
Jejunum	6.20	7.32	0.317	0.074
Ileum	7.16	7.75	0.28	0.358
Cecum	6.69b	7.79a	0.248	0.032
Colon	6.20	6.28	0.188	0.844
Lactic acid bacteria count (Log_{10} colony-forming units/g)				
Jejunum	7.26	8.03	0.208	0.074
Ileum	8.87	8.76	0.076	0.471
Cecum	8.07	8.53	0.241	0.305
Colon	7.55	7.56	0.084	0.993

SEM - standard error of the mean.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.
a,b - Average values followed by different lowercase letters in the row differ according to the F test at P≤0.05.

3.5. Densitometry and bone strength

Treatments did not affect (P>0.05) densitometry and bone strength (Table 8).

Table 8 - Additional dietary effect of calcitic seaweed in sow diets on piglet metacarpal bone densitometry and strength

Parameter	Treatment[1]		SEM	P-value[2]
	Control	Calcitic seaweed		
Maximum applied force (kgf)	26.22	22.17	3.379	0.557
Bone strength (N)	257.07	217.46	33.136	0.557
Area (cm^2)	3.12	3.33	0.151	0.524
Bone mineral content (g)	0.54	0.52	0.037	0.826
Bone mineral density (g/cm^2)	0.15	0.17	0.005	0.096

SEM - standard error of the mean.
[1] Addition of calcitic seaweed at a proportion of 0.4%.
[2] Significance level.

4. Discussion

4.1. Performance testing

In the present study, the effects of dietary CSW on pregnant and lactating sows, as well as the biological response of their litters were investigated. Previous studies on dietary CSW have shown inconsistent results in pigs (González-Vega et al., 2014; González-Vega et al., 2015; Santos et al., 2021).

The findings of the present study indicated that an organic calcium source can be added to sow diets without negatively affecting animal performance or reproduction, as also evidenced by Barrilli et al. (2017).

In addition, diets based on organic mineral sources improved reproductive performance of sows, especially litter size, which resulted in a greater number of live-born piglets compared with sows fed inorganic minerals (11.3 vs 10.6) (Peters and Mahan, 2008), which is in agreement with the present study (13.2 vs 11.6), respectively.

These results may be due to the similar effect of CSW on calcium and phosphorus metabolism compared to calcium carbonate (Schlegel and Gutzwiller, 2017). Additionally, CSW has been reported to be a more bioavailable calcium source (Barrilli et al., 2017), and it has a porous structure that promotes greater solubility compared with inorganic calcium, which increases calcium intestinal absorption (Melo et al., 2006).

Pregnant sows fed CSW showed improvements in farrowing performance. This could be explained by an increase in the bioavailability of calcium provided by CSW (Santos et al., 2021). During farrowing period, intramuscular calcium storage is extremely important for uterine contraction and fetal expulsion to reduce farrowing time and stillborns (Barrilli et al., 2017). Insufficient dietary calcium concentration affects requirements, imbalances minerals, increases farrowing time, and hence the number of stillborns (Gao et al., 2019).

The number of stillborns is affected by litter size, and longer farrowing can cause fetal death due to asphyxia (Rosa et al., 2014). In the present study, a reduction in stillborns was observed, even though sows fed CSW had heavier litters. Besides, a heavier litter could cause body weight loss in lactating sows, as they demand a greater milk supply and are more prone to distress (Martins et al., 2008). This was not observed in the present study because final body weight of lactating sows was not affected. However, a slight improvement was observed in body condition of sows fed CSW.

4.2. Blood metabolites

Although no difference between treatments was observed, calcium concentration on d 90 of gestation decreased. This is likely due to greater demand in late gestation for the end of piglet formation and milk production (Mellagi et al., 2013). Moreover, calcium total tract digestibility is altered in late pregnancy, which interferes with plasma calcium concentration (Lagos et al., 2019; Lee et al., 2019).

In late pregnancy, calcium supplementation is important to bring blood calcium concentration to proper levels. In the present study, CSW did not affect calcium concentration in sows. No physical alterations or evident deficiency factors were observed in animals subjected to this study. Gao et al. (2019) reported that maternal calcium supplementation at 15:00 h during late pregnancy and lactation can decrease stillborns and improve piglet growth performance by improving calcium concentrations.

In the present study, the average blood calcium concentration was 10.11 mg dL^{-1}; this value agrees with those previously reported (6.36 to 11.61 mg dL^{-1}) by Alexandre et al. (2005), for sows on d 60 of gestation fed dicalcium phosphate. Tan et al. (2016) studied the effects of dietary calcium availability on pregnant sows and reported that low dietary calcium concentration reduces serum calcium levels.

Lagos et al. (2019) reported that plasma calcium concentration in growing piglets was directly influenced by dietary calcium. However, literature is limited on studies evaluating different sources of calcium in sow diets, in which blood calcium concentration is analyzed in their litters. Santana et al. (2017) evaluated different calcium sources (limestone, monodicalcium phosphate, calcined bone meal, and oyster meal) in starter pig diets and found no differences in serum calcium concentration (≅10.60 mg dL^{-1}). This result was also reported by Santos et al. (2021), who studied dietary *L. calcareum* (2.35%) as calcium source for piglets. These authors observed no differences in plasma calcium concentration (≅11.12 mg dL^{-1}).

Lower plasma calcium concentration, compared with our results, was reported by Barrilli et al. (2017) in sows (days 14 and 21 of lactation) fed organic (≅5.5 mg dL^{-1}) and inorganic (≅5.6 mg dL^{-1}) calcium source. According to Santana et al. (2017) and Santos et al. (2021), plasma calcium concentration should range from 8 to 12 mg dL^{-1}. However, plasma calcium concentration is constant due to physiological mechanisms of mineral homeostasis (Santos et al., 2021). Therefore, calcium concentration must be considered along with other parameters to adequately measure the nutrient intake because calcium is closely regulated by mechanisms involving hormonal action (Lagos et al., 2019).

Reduction in blood glucose concentration is observed in late pregnancy and early lactation. As glucose is the main substrate for lactose synthesis, physiological and metabolic changes take place in sows' system during gestation to cope with subsequent lactation. Insulin decreases glucose absorption in other tissues, to provide the fetus (in late pregnancy) and the mammary epithelial cells to support milk production (Mellagi et al., 2010). An increased plasma urea concentration (PUC) could explain a reduction in glucose concentration. Urea synthesis increases energy consumption and hence reduces glucose that would be used for other purposes.

Plasma urea concentration was analyzed to verify the use of body protein and muscle catabolism in sows (Tokach et al., 2019). Although values are close to the established limits for the species, a reduction in PUC, as a result of increased feed intake, supports a lower body protein usage (Xue et al., 2012). Under nutritional deficiency or restoration of body minerals, breakdown of maternal protein tissues takes place to support fetal growth and/or reduced birth weight of piglets (Gaillard et al., 2019). A decrease in body protein storage mobilization can affect PUC to favor body condition score (Xue et al., 2012) and indicates an improvement in amino acids use to minimize body tissue mobilization (Soltwedel et al., 2006).

4.3. Milk production composition

At birth, piglets need energy from colostrum and milk for suckling, thermoregulation, and growth process. Thus, suckling limits survival and growth potential, hence animal performance (Barrilli

et al., 2017). Since milk synthesis occurs in the mammary epithelial cell and the number of these cells determines milk yield, sows must receive a balanced diet to produce more milk (Nuntapaitoon et al., 2020). Therefore, milk production and composition are directly related to feed intake, especially calcium (Gao et al., 2019). The more balanced the diet, the more nutrients are driven to milk, which possibly occurred in sows fed CSW, which has calcium and magnesium carbonate, more than 20 trace elements, and a porous structure that can retain water and nutrients. This favors digesta flow and a better nutrient absorption in gastrointestinal tract (Dias, 2000). Hence, it could explain the higher density and the increased nutrient content in milk.

Although milk production is supported by energy and protein balance, calcium requirements are directly affected by milk production (Tokach et al., 2019). Tan et al. (2016) reported that lower dietary calcium is related to milk calcium concentration. Thus, diet has an important role in milk/blood calcium concentration, and consequently, in milk composition. This was observed in a study conducted by Gao et al. (2019), who reported the importance of meeting dietary mineral requirements for lactating sows so as not to influence colostrum mineral composition. This also corroborates the study of Miller et al. (1994), who observed a effect of dietary calcium on milk composition and production and explained the role of calcium on protein stabilization in colloidal suspension.

Calcium demand for milk production requires some adaptive mechanism to maintain homeostasis. As lactation requires a large amount of calcium from the body and diet, the CSW diets may have positively influenced parathyroid hormone concentration, benefiting milk production (Barrilli et al., 2017).

4.4. Digestive tract contents pH, morphometry, and intestinal microbiota

During suckling period, as piglets adapt to the new diet, a series of physiological changes, such as pH, enzyme secretion, and intestinal motility, take place in their gastrointestinal tract. Thus, adequate nutrient digestion depends on the diet and intestinal structure and microbiota (Celi et al., 2017).

In the present study, maternal dietary CSW had no negative effects in the litters, such as changes in pH or intestinal morphometry alterations. Similarly, Almeida et al. (2012) reported that rats fed *L. calcareum* (30 and 120 mg per kg diet) did not show any gastric irritation nor change in pH. According to authors, this can be attributed to the high calcium carbonate concentration in the seaweed, and calcium carbonate stabilizes cell membrane by secreting bicarbonate, which regulates gastric pH.

On the other hand, González-Vega et al. (2014) tested different calcium sources and levels in growing pigs. They reported greater digesta pH in animals fed *L. calcareum* compared with those fed calcium carbonate. Authors attributed this effect to a reduction in mineral absorption in pigs fed *L. calcareum*, which could be explained by an increased concentration of soluble calcium in intestine (Schlegel and Gutzwiller, 2017) due to the higher solubility of CSW (Santos et al., 2021). This could have reduced the availability of minerals via formation of complexes among trace elements, hence changing gastrointestinal tract pH (González-Vega et al., 2014).

Piglet body weight gain is related to small intestine length and intestinal structures that affect nutrient absorption area (Celi et al., 2017). Intestinal villi height enlarges during lactation until weaning (Heim et al., 2015). These modifications are affected by diet, and the shortening of the villi has been indicative of enterocytes destruction, which reduces intestinal absorption area (Celi et al., 2017).

In the present study, we did not observe intestinal structural changes in piglets, although VH:CD ratio was slightly expressive in piglets from CSW-fed sows (Heim et al., 2015). An increase in paracellular absorption due to increased calcium in CSW could imply impairment of intestinal histology (Lagos et al., 2019).

However, Heim et al. (2014a) evaluated CSW supplementation in sows (10 g day^{-1}) and observed a positive effect on intestinal histology of piglets post-challenged with *Escherichia coli* K88. A similar effect was observed in weaning piglets (28 days of age) when sows received CSW-derived polysaccharides (10 g day^{-1}; Heim et al., 2015). Previous studies did not assess intestinal morphometry

of CSW-fed sows and calcium absorption site in gastrointestinal tract may shift according to calcium source (González-Vega et al., 2014).

There are no reports on the effect of maternal dietary calcium sources on the intestinal microbiota of litters. However, one of the most reported effects of calcium supplementation has been on LAB in hindgut (Blavi et al., 2018). However, in this study, we did not observe changes in LAB counts, although a slight increase in jejunal content was observed. This may be related to concentrations and sources of calcium, as well as the part of gastrointestinal tract (Mann et al., 2014).

The greater number of Enterobacteriaceae in the cecum of CSW-fed piglets may be associated with greater milk intake, which leads to an increase in endogenous substrate and a reduction in intestinal digesta flow (Dias, 2000). This is supported by the explanation that when endogenous substrates from small intestine enter the large intestine, changes in cecum microbiota can occur (Heo et al., 2013).

This greater Enterobacteriaceae count may be associated with higher milk production in sows fed CSW, which leads to a lower solid feed intake by the litters. In a study conducted by Choudhury et al. (2021), exclusively milk-fed piglets showed a change in microbiota composition when compared with those fed solid feed. Furthermore, intestinal microbiota needs to adapt to a new diet during suckling period (creep), which may result in small changes in microbial composition.

4.5. Densitometry and bone strength

Our results suggest that maternal dietary CSW was barely able to promote changes in the assessed bone traits in the litter. However, when piglets consumed high dietary *L. calcareum* (10 g calcium per kg of diet), there was a reduction in bone calcium concentration (Schlegel and Gutzwiller, 2017). Reduced bone mineralization was also observed when broilers fed diets containing 9 g calcium kg^{-1} supplied via *L. calcareum* (Walk et al., 2012).

Bones are the largest calcium storage in the body ensuring calcium homeostasis at a normal concentration in bones and extracellular environment (Gerlinger et al., 2019). Calcium plays an important role in health and bone structure (Tan et al., 2016). Piglet bone traits are related to plasma calcium concentrations (Santana et al., 2017), as dietary calcium intake or nutritional deficiency negatively influence bone strength (Lagos et al., 2019). Although calcium solubility in *L. calcareum* was greater than it is in inorganic sources (González-Vega et al., 2014), bioavailability of the calcium source and calcium interaction in bone metabolism (Schlegel and Gutzwiller, 2017) can compromise bone structure.

Similarly, Santana et al. (2017) reported no differences in growing pigs' metatarsals and reported that organic calcium sources are as effective as inorganic ones in maintaining bone mineral deposition for piglets. Likewise, in the study of Schlegel and Gutzwiller (2017), in which piglets fed different calcium sources, no differences metacarpal were found (7.9±1.0 kg of body weight), suggesting similar calcium metabolism.

Lagos et al. (2019) studied calcium influence in bone mineralization of growing pigs and reported that calcium limits bone deposition. However, they emphasized that calcium is also influenced by the concentration of phosphorus and this relationship favors the formation of hydroxyapatite crystals, hence, bone strength. Similar results were reported by González-Vega et al. (2014), who observed greater intestinal soluble calcium, promoting calcium phosphate or calcium phytate precipitation in the small intestine, with lower phosphorus availability and damage to bone mineral status.

Changes in calcium concentration promote metabolic disruptions and lower bone strength due to compromised bone resorption. Thus, these results suggest the amount of dietary CSW of the present study did not affect calcium concentration and allowed similar bone development compared with control group.

Altogether, maternal dietary CSW is an option considering nutritional requirements for animals at different phases. Adding CSW to pregnant and lactating sows diets improves some productive parameters without affecting performance and gastrointestinal tract and bone parameters of piglets.

5. Conclusions

This study showed that adding 0.4% calcitic seaweed in the diet of pregnant and lactating sows as an organic calcium source positively influences the number of piglets born alive and the percentage of stillborns. In addition, milk composition and production are also improved without affecting the piglets' biological response when the sows are fed 0.4% calcitic seaweed in the diet.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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