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The anatomical mammary gland position influences the weight gain and morphometry of piglets at weaning

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Abstract

The objective was to evaluate the performance of suckling piglets based on their choice of mammary gland. A completely randomized design was used, considering the position of the mammary glands pair and their respective piglets as experimental unit. The mammary gland position was considered for the treatments, being 1st, 4th and 7th pair. The ration and leftovers were weighed daily to obtain daily feed intake of the sow. On post-natal day (PND) 3 and 21, 80 ml of milk was collected to analyse crude protein, fat, lactose, non-fatty solids, mineral matter and total solids. On PND 3 and 21, the piglets were weighed to determine weight gain and estimate milk consumption. The piglets' longitudinal length and head circumference were measured in the same period and estimated body surface area/mass ratio. Blood samples from the sows and piglets were collected on PND 3 and 21 to analyse total cholesterol, triglycerides, total protein, fractions and glucose. There was an effect of mammary gland position on piglets at PND 21 suckling on the 7th pair, which had a 23.88 cm^2/kg greater body surface/mass ratio compared to those who suckled in the 1st pair. In turn, at PND 21, the piglets that suckled in the 1st pair presented higher weight, weight gain and milk consumption (0.685, 0.033 and 0.127 kg, respectively) than those that suckled in the 7th pair. Anterior mammary glands result in better productive performance and better chances of maintaining piglet homeostasis at PND 21.

Introduction

Genetic improvement in sow's maternal lines is aimed at reproductive characteristics such as high milk production, hyper prolificacy and more deposition of lean tissue in the progeny. However, complications arise with the increase in the litter size, such as greater variability in piglet weight and the birth of animals with low body weight (Moreira *et al*., 2020). In addition, piglets from large litters at birth face other complications, such as increased competition between piglets for mammary gland access.

Considering the body growth capacity of modern sows up to the third parity order, it is noted that the development of the udder in early calving can negatively affect the accessibility to the mammary glands. This fact can culminate in worse accessibility to the mammary glands by the piglets, considering that the upper line of the mammary glands during feeding will be at a high height and the lower one close to the ground, making it difficult to ingest milk (Vasdal and Andersen, 2012).

Although the number of mammary glands (teats) has increased, seeking to minimize the negative effects caused by large litters, the number of live-born piglets may still exceed the feeding capacity of the sows (Ocepek *et al*., 2016). Alexopoulos *et al*. (2018) highlighted that reports of females with more than 15 mammary glands have been cited in European studies, which suggest that genetic improvement in females should focus on enhancing the characteristics of the mammary system.

A series of factors intrinsic to the sow are related to the mammary gland development, such as genetics, age, hormone concentration, nutrition and management. Regarding this development, in addition to the factors mentioned, mammary growth during gestation is affected by the anatomical gland location, the anterior ones being usually the largest (Kim *et al*., 2013). In addition, further development of the mammary glands also occurs after birth in response to feeding (Ji *et al.*, 2006) from the stimulus promoted by the piglet. In this sense, the position and accessibility to the mammary gland can also influence the feeding process, and the increase in number of teats in modern lines of sows is a little-reported factor.

Thus, this study aimed to evaluate the effect of the anatomical position of the mammary glands on the piglets' performance from birth to post-natal day (PND) 21.

Materials and methods

Animals and facilities

The experiment was conducted with ten sows of commercial strain Topigs Norsvin Brazil® (TN70), at sixth parity order, at lactation phase. The sows were transferred from the gestation sheds to the maternity sheds at gestation day 105 and allocated to individual farrowing stalls. The sheds had negative room pressure and were provided with slatted floors, zinc feeders, pacifier-type drinkers for the sows and piglets.

The sows were fed 2.0 kg of diets during lactation until farrowing. After farrowing, 1.0 kg was offered; on the second day, 2.0 kg; on the third day, 3.0 kg; on the fourth day, 4.0 kg; on the fifth day, 5.0 kg; on the sixth day, 6.0 kg; on the seventh day, 7.0 kg; and from the eighth day until weaning, 8.0 kg of feed. Feeding was provided in five daily treatments, at 6:00, 10:00, 13:00, 16:00 and 22:00 h. The sows received water *ad libitum* during the experimental period.

The lactation feed was based on corn and soybean meal, according to the nutritional requirements of lactating sows (Topigs Norsvin, 2017; Table 1).

Experimental design

To determine the piglet's preference for the mammary gland (anterior, middle and posterior), observations of nursing behaviour were conducted during the first 3 days of lactation. The piglets were individually identified, and during this period, each piglet's preference for each mammary gland was observed. The 3-day observation period follows the premise of Rosillon-Warnier and Paquay (1984), who observed that after this period, the piglets established which mammary gland they would feed from throughout the entire lactation.

A completely randomized design was used, considering the position of the pair of mammary glands and their respective piglets as an experimental unit. The position of the mammary gland was considered for the treatments, being 1st (anterior), 4th (medial) and 7th pair (posterior).

Parameters evaluated in sows

Sows were weighed individually after farrowing and weaning. The collected data were used to verify body mobilization. The leftovers were weighed daily to obtain daily feed intake.

On PND 3 and 21, 80 ml of milk were collected by manual milking only of the pairs of mammary glands analysed using 10 IU of oxytocin injected into the atrial vein of the sow. Subsequently, the samples were conditioned at −20°C. The samples were analysed by infrared absorption to determine the contents of crude protein, fat, lactose, non-fatty solids, mineral matter and total solids (Bentley 2000®, Bentley Instruments Inc, Chaska, MN, USA).

Table 1. Centesimal and nutritional composition of feed for lactating sows

Ingredients	g/kg
Corn	597.3
Soybean meal	155.0
Extruded whole soybean	160.0
Sugar	50.0
Dicalcium phosphate	16.0
Limestone 0.38	6.0
Salt	5.0
Mineral and vitamin supplement[a]	4.0
L-lysine 0.98	3.2
L-threonine 0.99	2.5
DL-methionine 0.99	1.0
Total	**1000.00**
Metabolizable energy (MJ/kg)	13.963
Crude protein (g/kg)	186.4
Ether extract (g/kg)	58.2
Crude fibre (g/kg)	33.4
Calcium (g/kg)	8.9
Available phosphorus (g/kg)	5.2
Sodium (g/kg)	2.2
Total lysine (g/kg)	12.2
Methionine + total cysteine (g/kg)	7.2
Total threonine (g/kg)	9.5
Total tryptophan (g/kg)	2.4

[a]Cobalt (100.000 mg/kg), copper (10.000 g/kg), iron (20.000 g/kg), iodine (250.000 mg/kg), manganese (8.750.000 mg/kg), selenium (90.000 mg/kg), zinc (25.000 g/kg), vitamin A (2.500.000.000 IU/kg), vitamin D3 (450.000.000 IU/kg), vitamin E (7.620.000 IU/kg), vitamin K3 (625.000 mg/kg), vitamin B1 (550.000 mg/kg), vitamin B2 (1.250.000 mg/kg), vitamin B6 (750.000 mg/kg), vitamin B12 (7.500.000 mcg/kg), niacin (7.500.000 mg/kg), pantothenic acid (4.250.000 mg/kg), folic acid (750.000 mg/kg), biotin (100.00 mg/kg), choline (20.530 g/kg), B.H.T. (12.000 g/kg), 6-phytase (125.000.000 IU/kg).

Parameters evaluated in piglets

After birth, the litters were cross-fostered to maintain the same number of piglets compatible with the number of female mammary glands. On PND 3 and 21, the piglets were weighed individually to obtain the daily weight gain. The milk consumption of piglets was obtained by the equation suggested by Noblet and Etienne (1989):

$$\text{Estimated milk consumption (g/dia)} = (0.718 \times \text{DWG (g)} - 4.9)/0.19$$

where DWG is the piglet daily weight gain.

In the same period, the body and morphometric measurements of the piglets were measured. Measurement was performed from the middle portion of the skull, at the height of the ear base, to the first coccygeal vertebra to obtain the longitudinal length. The head circumference was obtained by measuring the perimeter just in front of the ears and below the mandible of the piglet using a tape measure. The relationship between the surface and the

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mass was calculated using the equations proposed by Meeh (Brody *et al*., 1928):

$$S = K \times W^{2/3}$$

where S is area in dm^2, K is 0.07 and W is body weight in kg.

$$\text{Surface area mass ratio } (dm^2/kg) = \text{body surface area } (dm^2)/\text{body weight (kg)}$$

Biochemical analysis

Blood samples from the sows and piglets were collected on PND 3 and 21 by puncture of the jugular vein, using vacuum blood collection tubes with sodium fluoride anticoagulant, to determine blood glucose, and without anticoagulant, with gel separator fraction, for biochemical analysis.

Blood samples without anticoagulant were separated into two aliquots of 500 µl and frozen until analysis. The following parameters were analysed: total cholesterol, triglycerides, total protein and fractions. Glucose was determined in plasma samples obtained after centrifugation of tubes containing sodium fluoride. Biochemical analyses were performed using commercial kits under enzymatic end-point colorimetric or kinetic methods in a semiautomatic biochemical analyser.

Environmental monitoring

The internal environment of the maternity rooms was monitored through a datalogger installed at half height of the body of the sows to collect temperature and relative humidity data every 5 min throughout the experimental period to characterize the thermal environment in which the sows and piglets were housed. During the experimental period, the room temperature ranged from 25.50 to 28.00°C, while the relative humidity was between 75 and 81%.

Statistical analysis

The data were submitted to the Shapiro–Wilk test at the level of 5% probability to verify data normality. The data with normal distribution were compared by the Tukey test of the analysis of variance at the level of 5% probability. The data with no normal distribution were normalized by the PROC RANK procedure of the SAS statistical package (9.3). The non-normalized data were compared by the Kruskal–Wallis test at the level of 5% probability.

Results

The average temperature was 26.5 ± 0.79°C and the average relative humidity was 78 ± 1.6%. The average thermal range was 2.37°C. The average relative humidity range was 5.94%. The highest average temperature was recorded at 13:00 h being 28.0°C and the lowest at 3:00 h being 25.6°C. The highest mean relative humidity was recorded at 19:00 h being 80.8% and the lowest at 13:00 h being 74.9% (Fig. 1).

The characterization of the performance and serum biochemical parameters of sows used in this study are reported in Table 2.

There was no effect ($P > 0.05$) of mammary gland position on the bromatological composition of milk (Table 3).

There was no effect ($P > 0.05$) of the position of the mammary gland on the biochemical serum profile of piglets at PND 3 and 21 (Table 4).

There was no effect ($P > 0.05$) of the position of the mammary gland on piglet morphometry, except ($P < 0.05$) for piglets at PND 21 which suckled in the 7th pair of mammary glands and had 23.88 cm^2/kg more surface/mass ratio compared to those who suckled in the 1st pair (Table 5).

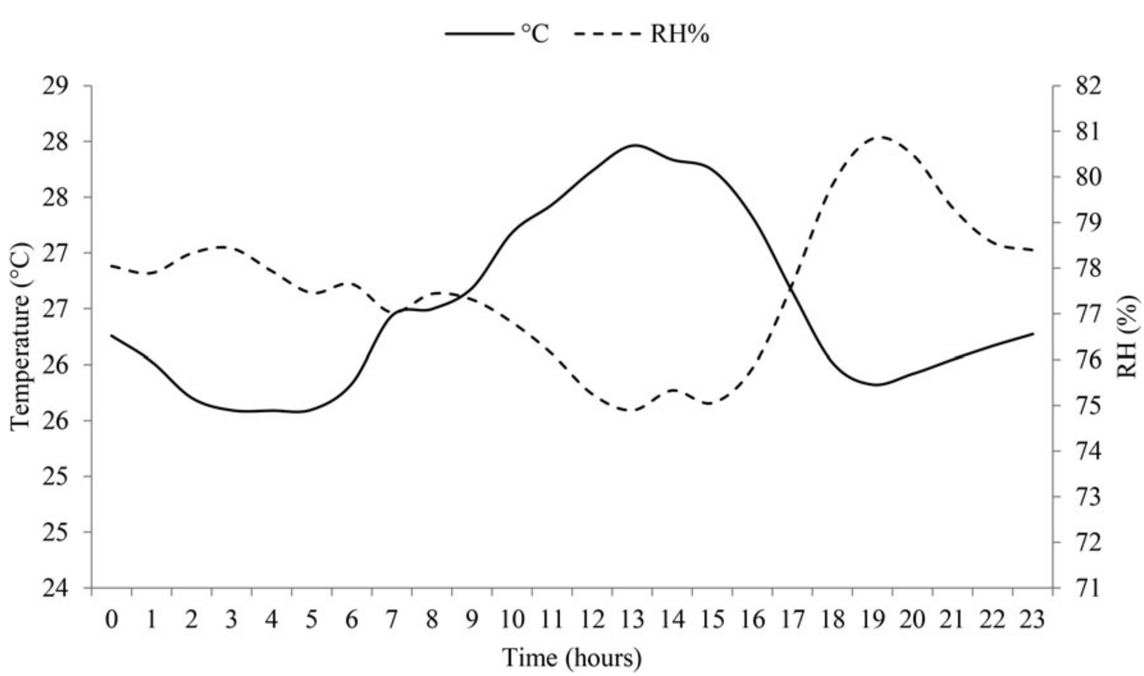

Figure 1. Average temperature (°C) and average relative humidity (%) during the experimental period.

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Table 2. Characterization of the performance and serum biochemical parameters of the sows

Parameters	Performance			
	Minimum	Maximum	Mean	Deviation
Mammary gland (n)	14	16	15.29	0.79
Lactation (days)	21.00	27.00	22.96	2.58
Postpartum weight (kg)	292.71	313.26	301.04	7.17
Weight at PND21 (kg)	235.00	296.50	267.67	23.66
Sow body mobilization (%)[a]	2.30	20.98	10.55	6.54
Daily feed intake (kg/day)	6.82	6.98	6.70	0.06
Milk production (kg/day)	5.92	12.33	10.02	1.80
Plasma biochemistry – 3rd day of lactation				
Total protein (g/dl)	5.30	13.10	8.53	2.41
Albumin (g/dl)	2.70	6.70	4.14	1.19
Cholesterol (mg/dl)	29.50	97.50	50.88	23.85
Glucose (mg/dl)	49.40	107.10	80.10	22.79
Creatine kinase (U/l)	113.30	1360.00	674.86	437.53
Aspartate aminotransferase (U/l)	19.60	35.90	24.46	5.96
Plasma biochemistry – 21st day of lactation				
Total protein (g/dl)	7.20	8.70	7.70	0.52
Albumin (g/dl)	2.50	4.60	3.80	0.74
Cholesterol (mg/dl)	7.00	90.50	50.06	24.78
Glucose (mg/dl)	74.30	125.40	97.30	15.80
Creatine kinase (U/l)	161.90	639.50	393.61	176.18
Aspartate aminotransferase (U/l)	20.80	47.40	29.78	10.35

[a]The relative change in the sow's weight, considering her initial weight (1 day after giving farrowing) and her final weight (weaning).

There was no effect ($P > 0.05$) of the position of the mammary gland on the weight of 3-day-old piglets (Table 6). In turn, at PND 21, the piglets that suckled in the 1st pair presented weight, weight gain and milk consumption values of 0.685, 0.033 and 0.127 kg, respectively, higher ($P < 0.05$) to those that suckled in the 7th pair (Table 6).

Table 3. Bromatological composition of milk

Parameters (g/kg)	1st pair	4th pair	7th pair	S.E.	*P* value
	3 days				
Fat	75	80	97	6.4	0.538
Crude protein	44.7	44.8	43.9	0.59	0.825
Lactose	50.1	48.5	47.9	0.94	0.393
Non-fat solids	108.0	106.9	106.6	0.81	0.770
Total solids	183	187	204	6.2	0.167
	21 days				
Fat	70	90	73	4.8	0.252
Crude protein	45.3	46.1	45.4	0.77	0.915
Lactose	52	48	51	1.5	0.432
Non-fat solids	111	109	111	2.4	0.900
Total solids	180	199	185	4.5	0.181

Discussion

The sows were not in thermal comfort since the Topigs Norsvin® females manual recommended temperature for the category is between 16 and 22°C. The values found in the present study for the air's relative humidity exceed those proposed by the literature, which suggest values between 40 and 70% (Ribeiro *et al.*, 2018). The data may suggest that the females encountered difficulties performing heat losses since pigs perform homeothermic exchanges mainly through evaporation. Humidity limits evaporative losses through respiration when the temperature and relative humidity of the air are high, which can culminate in decreased appetite and, consequently, productivity (Cecchin *et al.*, 2017).

Regarding hyperprolific sows, the probability of them entering a negative energy balance is much higher due to the high metabolic demands during gestation and also in lactation phase (Costermans *et al.*, 2020). Therefore, to meet milk production and maintenance, sows must have their energy, protein and mineral requirements met. In the present study, there were no significant mobilizations of the sow for these aspects, data that are consistent with the data found by dos Santos *et al.* (2023). Sows under heat stress reduce feed intake in an attempt to lower body temperature, which can lead to body mobilization and

Table 4. Biochemical serum profile of piglets as a function of mammary gland position

Parameters	1st pair 3 days	4th pair	7th pair	S.E.	*P* value
Total protein (g/dl)	8.3	9.3	8.2	0.44	0.730
Albumin (g/dl)	4.4	4.3	4.0	0.28	0.729
Cholesterol (mg/dl)	151	133	154	7.6	0.455
Glucose (mg/dl)	168	176	157	5.2	0.352
Creatine kinase (U/l)	362	477	127	81.3	0.215
Aspartate aminotransferase (U/l)	28	33	32	1.7	0.417
	21 days				
Total protein (g/dl)	5.7	5.6	5.9	0.12	0.454
Albumin (g/dl)	3.4	3.4	3.4	0.11	0.888
Cholesterol (mg/dl)	128	105	107	9.6	0.461
Glucose (mg/dl)	160	144	129	6.8	0.310
Creatine kinase (U/l)	499	1046	540	180.4	0.948
Aspartate aminotransferase (U/l)	35	40	32	2.3	0.439

may affect piglet weight gain due to reduced milk production by the sows.

Biochemical analyses were used to evaluate animals' nutritional, clinical, metabolic and productive states. They can be influenced by many variables, such as age, lineage, diet, disease and stage of gestation and lactation, being able to indicate levels of muscle injury, repair of reproductive tissues and the catabolism of body reserves (Diaz Gonzalez and da Silva, 2017; Padilha *et al*., 2017; Rempel *et al*., 2018). The values found in the present study for serum

Table 5. Morphometry of piglets as a function of mammary gland position

Parameters	1st pair 3 days	4th pair	7th pair	S.E.	*P* value
Longitudinal length (cm)	29.5	29.6	28.9	0.36	0.737
Head circumference (cm)	23.5	23.6	23.3	0.18	0.944
Surface/mass ratio (cm^2/kg)	547	548	570	6.3	0.291
	21 days				
Longitudinal length (cm)	44.2	43.6	42.4	0.70	0.620
Head circumference (cm)	30.7	30.7	30.0	0.33	0.411
Surface/mass ratio (cm^2/kg)[a]	435	446	459	5.1	0.035

[a]Head circumference was applied to the piglets at 3 days as a co-variable ($r = -0.66$ and P value ≤ 0.001).

Table 6. Piglet performance as a function of the position of the mammary glands

Parameters	1st pair	4th pair	7th pair	S.E.	*P* value
OBS	20	20	14		
Weight at 3 days (kg)	2.2	2.2	1.9	0.07	0.296
Weight at 21 days (kg)[a]	5.3	4.9	4.6	0.21	0.025
Daily weight gain (kg/day)[b]	0.1	0.1	0.1	0.01	0.044
Estimated milk consumption (kg/day)[c]	0.5	0.5	0.4	0.03	0.043

[a]Head circumference was applied at 21 days of age as a co-variable ($r = 0.87$ and P value ≤ 0.001).
[b]Longitudinal length was applied at 21 days of age as a co-variable ($r = 0.77$ and P value ≤ 0.001).
[c]Longitudinal length was applied at 21 days as a co-variable ($r = 0.77$ and P value ≤ 0.001).

biochemical parameters are consistent with those observed by dos Santos *et al*. (2023), who observed adequate protein consumption by the mothers, which led them to sustain their milk production and maintenance. The same authors also found no catabolic mobilizations of the sow through the non-alteration of creatine kinase. The probability of finding animals with alterations in the biochemical profile is low, considering that the animals of the present study were inserted in a pig breeding system with a balanced diet, well-established sanitary programme and favourable climate.

As in the present study, Atwood and Hartmann (1992) found no significant changes in protein or lactose levels evaluating milk composition of sows' anterior and posterior teats during lactation. However, these authors found a higher fat concentration from the milk from the anterior teats. It is necessary to ensure that the sow has a large and uniform milk production throughout the mammary gland to ensure the uniformity of the piglets during weaning, given that, from birth to 4 weeks of life, the primary feed source of the piglets is the sow's milk (Nielsen *et al*., 2001).

In addition, the milk must be equalized uniformly, considering that the more significant weight variations result in preference of the heavier piglets to the anterior teats, which have higher milk production, and leave the posterior teats, where milk production is lower, to the lighter piglets (Kim *et al*., 2000; Devillers *et al*., 2016; Rendón del Águila et al., 2017; de Paula *et al*., 2019), thus resulting in great milk variability, and a reduction in the post-weaning survival rate (Farmer and Edwards, 2022).

The litters studied were in similar conditions; they were cross-fostered with the same number of piglets as in those in which there was no significant difference in weight. In this sense, the teats' position did not affect the piglets' biochemical serum components since no changes were observed in the estimated production and composition of the sow's milk. Wang *et al*. (2021) stated that the values of serum biochemical parameters of lactating piglets continuously change until weaning while weaning leads to changes in many processes of nutritional metabolism. Kabalin *et al*. (2017) determined that neonatal piglets are neurologically mature but physiologically immature.

Parallel to this, the hepatic system of this animal category is not well developed at birth, and we can expect divergences in the maturation of the glycogenolytic and gluconeogenic pathways, which consequently results in limitations in the synthesis of glucose and the stability of glycaemia. However, lactating piglets, which have greater mass at birth, can be considered more mature physiologically and can achieve immunological competence earlier. The synthesis and maintenance of some serum components is also established earlier.

Morphometric parameters are indicators of piglets' survival capacity since they are directly linked to their ability to thermoregulate, which can also affect their performance. Suckling piglets with a higher surface-to-mass ratio may be more likely to suffer from hypothermia as they lose heat more easily to the environment. Piglets with better morphometric indices also have better growth rates and greater ability to compete for the mammary glands (Huting *et al.*, 2018; Tucker *et al.*, 2022).

Kim *et al.* (2000) found that piglets prefer the anterior mammary glands. This fact can be justified by two factors: the position of these glands offers greater safety and milk production, and piglets are encouraged to be closer to the head of the sow, attracted by its vocalization during feedings (Devillers *et al.*, 2016). In turn, milk production can be explained by the anterior mammary glands having arteries with diameters larger than the caudal arteries (de Paula *et al.*, 2019). The results obtained in the present study indicate a positive relationship between the access of the piglets to anterior mammary glands and their performance, showing that dominance is influenced by the piglets' weight, as also observed by Gomboc *et al.* (2023).

Nielsen *et al.* (2001) observed that the DNA and RNA content are higher in the anterior mammary glands compared to the posterior glands. This pattern may be related to the difference in milk production between the mammary glands. In addition, the same authors noted that the growth rate of suckling piglets in the anterior glands was higher than in the posterior glands. Skok and Škorjanc (2013) verified a positive correlation between milk consumption and weaning weight. In the present study, based on the estimated milk consumption by the piglets, it can be inferred that those that suckled from the anterior mammary glands ingested more milk, had a higher weaning weight and exhibited a lower surface area/mass ratio.

Conclusion

Anterior mammary glands result in better productive performance and better chances of maintaining piglet homeostasis at piglets from birth to PND 21.

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