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ANIMAL PRODUCTION

Guanidinoacetic acid supplementation improves reproductive performance of gilts during gestation and lactation

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ABSTRACT: This study evaluated dietary supplementation of guanidinoacetic acid (GAA) during gestation and lactation of high-prolific gilts on reproductive performance, milk amino acid profile and suckling piglets' performance. A total of 53 gilts were distributed among 2 dietary treatments (Control diet (CON) and a GAA diet, CON + 1,0 g/kg of GAA (GAA)) in a completely randomized experimental design during gestation phase with 27 and 26 replicates respectively. During the lactation phase, according to the previous treatments and the use or not of GAA in the diets, the gilts were distributed in a 2 x 2 factorial design. The number of gilts were randomized design in four treatments: A control diet during gestation and lactation (CON-CON); a control diet during gestation and GAA during lactation (CON-GAA); GAA during gestation and CON diet during lactation (GAA-CON); and GAA during gestation and lactation (GAA-GAA). Gilts per treatment consisted of 13, 14, 13, 13 respectively. GAA supplementation for gilts improved reproductive traits and tended to increase the total number of piglets born alive ($P = 0.059$). The use of GAA during lactation increased daily milk production throughout this phase ($P = 0,.01$), and improved milk amino acid content on d 7 of lactation ($P < 0.5$). Consequently, GAA supplementation in gestation and lactation impacted on lactation performance in first parity gilts, improving daily litter and piglet weight gain ($P = 0,.05$), as well as litter and piglet weaning weights ($P = 0.05$). In conclusion, the use of GAA improves reproductive performance.
Key words: arginine, born alive, creatine, gilts, lactation.

A suplementação de ácido guanidinoacético melhora o desempenho de leitoas durante a gestação e lactação

RESUMO: O objetivo deste estudo foi avaliar a suplementação dietética do ácido guanidinoacético (GAA) durante a gestação e lactação de matrizes hiperprolíficas quanto ao desempenho reprodutivo, perfil de aminoácidos do leite e desempenho dos leitões na maternidade. Um total de 53 matrizes foram distribuídas entre dois tratamentos (dieta Controle (CON) e dieta COM + 1,0 g/kg de GAA (GAA)) em um desenho experimental totalmente aleatorizado durante a fase de gestação, com 27 e 26 animais, respectivamente. Durante a fase de lactação, de acordo com os tratamentos anteriores e o uso ou não de GAA nas dietas, as matrizes foram distribuídas em um desenho fatorial 2 x 2. As matrizes foram alocadas aleatoriamente em quatro tratamentos: uma dieta controle durante a gestação e lactação (CON-CON); uma dieta controle durante a gestação e GAA durante a lactação (CON-GAA); GAA durante a gestação e dieta CON durante a lactação (GAA-CON); e GAA durante a gestação e lactação (GAA-GAA). O número de matrizes por tratamento foram de 13, 14, 13 e 13, respectivamente. A suplementação com GAA para matrizes melhorou as características reprodutivas e tendeu a aumentar o número total de leitões nascidos vivos ($P = 0,059$). O uso de GAA durante a lactação aumentou a produção diária de leite ao longo dessa fase ($P = 0,001$) e melhorou o conteúdo de aminoácidos do leite no 7º dia de lactação ($P < 0,05$). Consequentemente, a suplementação com GAA na gestação e lactação impactou no desempenho da lactação em matrizes de primeiro parto, melhorando o ganho de peso diário da leitegada e dos leitões ($P = 0,005$), assim como os pesos de desmame da leitegada e dos leitões ($P = 0,05$). Em conclusão, o uso de GAA melhorou o desempenho reprodutivo de matrizes jovens.
Palavras-chave: arginina, nascidos vivos, creatina, leitoas, lactação.

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INTRODUCTION

Hyperprolific sow's present specific challenges that are directly linked to the balance between the sow's nutritional requirements and its reproductive capacity. They have larger litter and require greater amounts of nutrients to support fetal growth and maintain the sow's health. If these requirements are not attended, a decline in piglet quality or even fertility problems may occur.

Creatine is a molecule that plays a role in the production and utilization of energy in cells, especially those with high energy demands, such as the reproductive system (ROSA et al., 2018). Creatine is synthesized in the body from three amino acids:glycine, arginine, and methionine. GAA is a direct precursor of creatine and is converted into creatine in a two-step process, with the help of the enzymes guanidinoacetate methyltransferase and the molecule Sadenosylmethionine (SAM) (WU et al., 2017). The mechanism of action of guanidinoacetic acid (GAA) involves its role in the creatine biosynthesis pathway, which leads to improved energy metabolism and enhanced fertility of gilts.

The increased availability of creatine due to GAA supplementation leads to faster energy metabolism in the reproductive system of gilts, which increases energy metabolism results in increased follicular development and oocyte quality, and can increase embryonic survival rates (MATEO et al., 2008). Additionally, GAA supplementation has been shown to increase serum concentrations of insulin-like growth factor-1 (IGF-1), which is involved in ovarian function and follicular development (WU et al., 2017).

Furthermore, the supplementation of GAA, as a direct precursor of creatine, has the potential to preserve arginine in a metabolic pathway, enabling arginine to participate in the metabolic processes. Arginine has been identified as an important amino acid in the vascularization and development of the placenta in gilts, particularly during early gestation (WU et al., 2007).

There is a correlation between arginine and developing fetuses, due to the nitric oxide and polyamines involvement, and their effects during critical stages of gestation, such as angiogenesis, placental blood circulation, and embryogenesis (ABREU et al., 2013; RODRIGUES et al., 2021). In lactating gilts, the higher concentration of arginine can increase blood flow and nutrients supply to the mammary gland, due to the greater synthesis of nitric oxide in the endothelial cells of blood vessels (WU & MEININGER, 2003). We hypothesized that the use of GAA may improve the reproductive performance of gilts and litter size, but the effect may be dependent on the supplementation period. Therefore, this study evaluated the effect of dietary GAA supplementation on the performance of gilts during gestation and lactation phases, as well as on the performance of suckling piglets.

MATERIALS AND METHODS

The study was conducted on a commercial farm, located in the Southern region of Brazil, in the state of Santa Catarina.

Animals and experimental procedure
Gestation phase

A total of 53 gilts (Topigs Norsvin TN70), with 250 days old were used. Gilts were distributed in a completely randomized experimental design among 2 dietary treatments. All gilts were within the same range of age, body weight and backfat thickness 2400 h after insemination. The gilts were allocated to one of the two treatments: CON, a control diet, with 27 gilts, and GAA, the control diet supplemented with GAA 96% (i.e., 1 g/kg of GAA), during the gestation phase (Table 1) with 26 gilts per treatment. Each sow was considered an experimental unit.

The gilts remained in the experimental treatments from d 1 post insemination until farrowing (115 d). The gilts were housed individually in gestation crates, received a controlled amount of feed, and had ad libitum access to water. The differences in ambient temperature, relative humidity (RH), and the photoperiod followed similarly to outdoor conditions. Ambient temperature and RH were measured daily recorded (1 measurement every 5 minutes) in the barns, with the datalogger connected to a probe (Didai Tecnologia Ltda, Campinas, Brazil) at 1 m above the ground.

Gestation experimental diets were isoenergetic, isonitrogenous, and formulated to meet gilts' nutritional requirements (ROSTAGNO et al., 2017; Table 1). Fresh feed was distributed once per day at 07:00 h after the refusals from the previous day were collected. Feed consumption was determined as the difference between feed allowance and the number of refusals collected the next morning. For DM content measurement, one sample of feed was collected every day. Subsequent samples are analyzed using pools of samples stored at 4 °C for the remainder of the day.

During gestation, feeding levels were set at 2.2 kg d^{-1} in the first third of gestation (insemination until 30 d), 2.1 kg d^{-1} in the second third of gestation (31d to 84 d), and at 2.75 kg d^{-1}

Table 1 - Gestation experimental diets composition (as-fed basis).

	Gestation	
Ingredients (kg)	CON	GAA
Corn (7.8% CP)	565.82	565.82
Soybean meal (45% CP)	205.00	205.00
Maize Starch	1.00	-
Wheat middling	200.00	200.00
Dicalcium phosphate	8.28	8.28
Limestone (36%)	13.05	13.05
Salt	4.55	4.55
GAA[1]	0.00	1.00
Premix Min./Vit.[2]	2.30	2.30
Total	1,000.00	1,000.00
Analyzed composition CP (%)	16.14	16.10
CF (%)	4.27	4.35
Calculated ME (MJ)	12.00	12.01
Calcium (%)	0.80	0.85
Avail. phosphorus (%) Amino acids	0.27	0.31
SID Lysine (%)	0.85	0.83
SID Met. (%)	0.22	0.27
SID Met. + Cyst. (%)	0.53	0.54
SID Threonine (%)	0.62	0.66
SID Tryptophan (%)	0.18	0.17
SID Arginine (%)	0.98	0.99
SID Valine (%)	0.75	0.77
SID Cysteine (%)	0.32	0.34
SID Histidine (%)	0.41	0.41
SID Isoleucine (%)	0.66	0.67
SID Leucine (%)	1.48	1.53
SID Phenylalanine (%)	0.79	0.75
SID Glycine (%)	0.79	0.85
SID Proline (%)	1.13	1.19
SID Serine (%)	0.74	0.77
SID Alanine (%)	0.91	0.93
SID Glutamine (%)	2.75	2.77
GAA (%)	-	0.091
Sodium (%)	0.22	0.24

CP – Crude Protein; CF – Crude Fiber; SID-Standardized ileal digestible, GAA – Guanidinoacetic acid. [1]Contained min 96% GAA. [2]Contents in 1 kg of feed: Iron, 100 mg; Copper, 10 mg; Cobalt, 1 mg; Manganese, 40 mg; Zinc, 100 mg; Iodine, 1.5 mg, Selenium, 300 mg and vehicle q.s .Contents in 1 kg of feed: Vit A - 8000 IU; Vit D3 - 1200 IU; Vit E - 20 IU; Vit K3 - 2 mg; Vit B1 - 1 mg; Vit B2 - 4 mg; nicotinic acid – 22 mg; pantothenic acid - 16 mg; Vit B6 - 0.50 mg; Vit B12 - 0.020 mg; folic acid - 0.4 mg; biotin - 0.120 mg; choline - 400 mg; and antioxidant – 30 mg. CON – Control diet; GAA – Control diet supplemented with GAA.

in the final third of gestation (85d to 109 d). From day 110 until farrowing gilts went to the farrowing crates and received 2.0 kg d^{-1} of the feed of their respective treatment. The gilts were subjected to measurements for body weight using a digital scale (Líder Balanças Ltda., Mod. LD 2000E, Araçatuba, SP, Brazil) and backfat thickness was determined at P2 location (65 mm from the dorsal line) through ultrasound assessment (Renco Lean-Meater, Renco Corporation, Minneapolis, USA) at 2400 h post insemination; and subsequently, on day 84, day110 and 2400 h post-farrowing. During the farrowing period, the following litter parameters were collected: number of live piglets, stillborn and mummified, and variation in birthweight within-litter. Immediately after birth, each piglet was weighed using a digital scale (Líder Balanças Ltda., Mod. B150, Araçatuba, SP, Brazil).

Lactation Phase

At 110 days of gestation, the same 53 gilts were moved to farrowing crates, where they remained until weaning. The lactation period lasted for a total of 26 days. Animals were distributed in a factorial 2 x 2 experimental design. The gilts were distributed following the previous treatment applied during gestation (CON and GAA), and according to body weight and backfat thickness after farrowing. The gilts were allocated to one of the four treatments: a control diet during gestation and lactation (CON-CON); a control diet during gestation and the control diet supplemented with GAA 96% (i.e., 1 g/kg of GAA) during lactation (CON-GAA); control diet supplemented with GAA 96% during gestation and the control diet during lactation (GAA-CON); and control diet supplemented with GAA 96% during gestation and lactation (GAA-GAA). The number of gilts per treatment was 13, 14, 13, 13 respectively, each gilt was considered a unit experimental.

Gilts were kept on the treatments until weaning. The gilts received the experimental diets on the day after farrowing, then all the gilts were weighed and the thickness of the backfat was measured. Then all females were subjected to a gradual feed intake stimulation until day 7 after parturition, with 2 kg on day 1 and reaching 8 kg d^{-1} on day 7, increased by 1 kg per day. After 7 d, gilt was fed *ad libitum.* Piglets did not receive creep feeding during the lactation period.

Lactation experimental diets were isoenergetic, isonitrogenous, and formulated to meet gilts´s nutritional recommendations (ROSTAGNO et al., 2017; Table 2). Feed was offered early in the morning (i.e., between 07:00 h and 08:00 h) after refusals were collected. Feed consumption was calculated by the difference between the feed supply and the leftovers the next morning. Each day, a sample of feed and feed refusals was collected for DM and successive samples were pooled and stored at 4 °C for later analysis.

After the litter birth variables were noted, the litter was standardized to 13 - 14 piglets at 48:00 h post farrowing. Litter performance was also evaluated at birth, 24:00 h, 48:00 h, 7 day and at weaning. On the day before weaning, gilts were allowed to eat 4 kg of feed this was to equalize consumption for all gilts to determine weaning weight o. At weaning, gilts were again weighed and backfat was measured, as described for gestation phase, and then moved to a breeding facility and were presented twice daily to a mature boar to detect the onset of standing estrus. During this period, all gilts were fed with 3.5 kg d^{-1} of their respective lactation diet.

For the milk samples, the following protocol was applied to mimic a suckling event. Piglets were separated from the sow ‘s after suckling and 45 to 50 min later the gilts were milked (SILVA et al., 2009). For that, intravenous injection of 10 i.u. oxytocin into an ear vein on d 7 and d 18 of lactation, and samples were manually collected with 10 replicates from each functional mammary gland of each sow, from a pre-determined subsample of 10 gilts per treatment. The amount of milk collected (150 to 200 mL) was close to the estimated milk production during one suckling between farrowing and day 20. Samples were stored at -20 °C, immediately after collection. At the end of the experiment, all samples were lyophilized. The amino acids contents in feed and milk samples were analyzed by Evonik Operations GmbH.

Amino acid analyses

Amino acid contents were determined by ion-exchange liquid chromatography with post-column derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with sodium metabisulfite (COMMISSION DIRECTIVE, 1998). Amino acids were liberated from the protein by hydrolysis with 6 NHCL for 24:00 h at 110 °C and AA were quantified with the internal standard method by measuring the absorption of reactive products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide for 2000 h at 110 °C (COMMISSION DIRECTIVE, 2000). The supplemented feed additive GAA was measured by ion chromatography coupled with ultraviolet detection (IC-UV). Detailed information on the methodology used to obtain the results can be found in European Reference Laboratory guidelines <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluationreports>.

Calculations

The average of the maximum and minimum temperatures, the average and variance of the daily ambient temperatures and relative humidities for all the days of the experiment were calculated. Protein, lipid, and energy deficits in the lactational period were estimated as the difference between the calculated values determined at weaning and farrowing, according to the equations of DOURMAD et al. (1998) and described by SILVA et al. (2017). The average daily milk production was based on the growth rate and size of the litters during lactation, according to the equations of Noblet and Etienne

Table 2 - Lactation experimental diets composition (as-fed basis).

Ingredients (kg)	CON	GAA
Corn (7.8% CP)	670.26	670.26
Soybean meal (45% CP)	250.00	250.00
Soybean oil	40.40	40.40
Dicalcium phosphate	15.00	15.00
Limestone (36%)	9.50	9.50
NaCl	4.99	4.99
Maize Starch	1.00	-
GAA[1]	0.00	1.00
L-Lysine HCL	3.01	3.01
DL-Methionine	0.46	0.46
L-Threonine	1.08	1.08
L-Tryptophan	0.30	0.30
Premix Min./Vit.[2]	4.00	4.00
Total	1.000,00	1.000,00
Analyzed composition CP (%)	22.21	22.22
CF (%)	2.83	2.81
Calculated ME (MJ)	13.82	13.81
Calcium (%)	0.85	0.87
Avail. phosphorus (%) Amino acids	0.40	0.40
SID Lysine (%)	1.37	1.47
SID Met. (%)	0.27	0.32
SID Met. + Cyst. (%)	0.65	0.68
SID Threonine (%)	0.93	0.92
SID Tryptophan (%)	0.14	0.17
SID Arginine (%)	1.38	1.38
SID Valine (%)	0.97	0.99
SID Cysteine (%)	0.34	0.32
SID Histidine (%)	0.55	0.57
SID Isoleucine (%)	0.83	0.81
SID Leucine (%)	1.77	1.79
SID Phenylalanine (%)	1.00	1.03
SID Glycine (%)	1.05	1.02
SID Proline (%)	1.27	1.32
SID Serine (%)	1.12	1.14
SID Alanine (%)	1.07	1.05
SID Glutamine (%)	3.45	3.38
GAA (%)	-	0.089
Sodium (%)	0.23	0.22

CP -Crude Protein; CF -Crude Fiber; SID-Standardized ileal digestible, GAA- Guanidinoacetic acid. [1]Contained min 96% GAA. [2]Contents in 1 kg of feed: Iron, 100 mg; Copper, 10 mg; Cobalt, 1 mg; Manganese, 40 mg; Zinc, 100 mg; Iodine, 1.5 mg, Selenium, 300 mg and vehicle q.s. Contents in 1 kg of feed: Vit A - 8000 IU; Vit D3 - 1200 IU; Vit E - 20 IU; Vit K3 - 2 mg; Vit B1 - 1 mg; Vit B2 - 4 mg; nicotinic acid – 22 mg; pantothenic acid - 16 mg; Vit B6 - 0.50 mg; Vit B12 - 0.020 mg; folic acid - 0.4 mg; biotin - 0.120 mg; choline - 400 mg; and antioxidant – 30 mg. CON – Control diet; GAA – Control diet supplemented with GAA.

(1989), based on the average daily piglet weight gain (g/d), the average number of piglets, and the milk dry matter (19%) Milk Protein (kg/d) = ([0.718×Average daily gain-4.9] × Number of piglets)/ 0.19. The data were analyzed differently during the gestation and lactation periods. The gestational period data were analyzed for normality and homoscedasticity of variances and later submitted to analysis of variance (ANOVA), where the F test was conclusive at a significance level of $P < 0.1$ of probability. It was considered a tendency when $P > 0.05$ and $P < 0.1$. The variables number of stillborn piglets and number of mummified piglets were analyzed using the Kruskal-Wallis's test. The experiment consisted of two phases. In the first phase, during the gestational period, the animals were divided into two groups: those treated with GAA and those not treated with GAA. The second phase was a factorial experiment, where the animals from the gestation period were again divided into two groups: those receiving GAA and those not receiving GAA. The following treatment combinations were formed: Gestation with GAA and lactation with GAA; Gestation with GAA and lactation without GAA; Gestation without GAA and lactation with GAA; and Gestation without GAA and lactation without GAA, and the means were compared using the Tukey test at 0.05 of probability. The number of gilts that returned to estrus before and after 5 d post-weaning were compared using a $\chi 2$ test (R's FREQ procedure). The number of piglets at birth was tested as a covariate for the performance variables of gilts and the performance of the litter during lactation, but it was not significant. Statistical analysis was performed using the R software (version 3.4.4, 2017, R CORE TEAM 2021).

RESULTS

The average maximum 29.1 °C and minimum 17.8 °C temperatures measured during the experimental period.

Gestation phase

The GAA supplementation tended to increase the total number of born (15.74 vs. 16.65, respectively for CON and GAA; P = 0.072; Table 3), born alive (13.85 vs. 14.86, respectively for CON and GAA; P = 0.059; Table 3) and the average litter birth weight (22.65 vs. 23.42 kg, respectively for CON and GAA; P = 0.067; Table 3).

Lactation phase

GAA-GAA treatments showed the highest (P = 0.019) voluntary intake of the gilts. The lactation body weight loss was not influenced (P > 0.10) by the treatments (Table 4). The chemical composition of body weight loss did not differ between treatments as well (P > 0.10; Table 4).

The litter size and the average weight of the piglets after cross-fostering (48 h) and at weaning did not differ between treatments (P > 0.10). Effects of interactions were observed, where GAA-GAA fed gilts presented a higher daily litter gain (P = 0.001), litter weaning weight (P = 0.005), piglet weaning weight (P = 0.001), piglet weight gain (P = 0.003) and average daily milk production (P = 0.001) in comparison to the other treatments.

An interaction between treatments for the levels of lysine (P = 0.032; Table 5) and threonine (P = 0.026; Table 5) in milk composition on d 7 was observed. Gilts supplemented with GAA during both gestation and lactation showed improved levels of both amino acids in milk. There was also a tendency (P < 0.10) for the interaction between treatments for methionine + cysteine, phenylalanine, glycine and alanine. The use of GAA influenced (P < 0.05; Table 5) the amino acids methionine, tryptophan, arginine, isoleucine, leucine, valine, histidine, serine, proline, Asx and Glx in milk composition on d 7 of lactation, without the influence of gestation phase.

Table 3 - Effects of dietary guanidinoacetic acid (GAA) supplementation during gestation on gilt reproductive performance at farrowing (LSmeans).

Treatments	CON	GAA	SEM[1]	P-Value
Number of gilts	27	26	-	-
Initial Body Weight, kg	187.48	190.27	0.287	0.952
Initial backfat thickness, mm	12.56	13.07	0.363	0.735
Total born, n	15.74	16.65	0.340	0.072
Total born alive, n	13.85	14.86	0.360	0.059
Mummified, n	0.55	0.43	0.130	0.652
Stillbirth, n	1.33	1.26	0.270	0.863
Average piglet weight at birth, kg	1.50	1.45	0.020	0.337
Average litter weight at birth, kg	22.65	23.42	0.580	0.067

[1]Standard error of the mean. CON = Gilts fed with the control diet during gestation; GAA = Gilts fed with the control diet supplemented with guanidinoacetic acid (GAA).

Table 4 - Effects of dietary guanidinoacetic acid (GAA) supplementation for gestating and.

	--------------Gestation's Treatment*------------							
	--------------------CON		GAA--------------------					
Parameters	--------------Lactation's Treatment*------------					--------------P-Value²------------		
	CON	GAA	CON	GAA	SEM¹	GT	LT	G x L
Number of gilts	13	14	13	13	-	-	-	-
Initial body weight, kg	193.80	200.70	194.70	199.40	0.526	0.125	0.536	0.369
Initial backfat thickness, mm	14.90	15.30	1.90	16.30	0.234	0.332	0.224	0.421
Lactation period, d	25.80	26.50	26.40	26.00	0.300	0.260	0.450	0.350
ADFI (d 1 until weaning), kg d^{-1}	3.98^{b}	4.05^{b}	3.83^{b}	4.17^{a}	0.070	0.600	0.010	0.090
Total feed intake (d 1 until weaning), kg	103.10	107.40	101.10	108.90	2.300	0.350	0.090	0.060
Body weight loss, %	8.60	8.20	7.70	8.90	1.200	0.870	0.670	0.590
---------Chemical composition of body loss³---------								
Protein, kg	1.75	1.33	1.45	1.60	0.560	0.690	0.890	0.790
Lipids, kg	6.66	7.39	6.17	7.7	0.700	0.640	0.300	0.400
Energy, MJ	469.00	495.00	438.00	520.00	43.000	0.760	0.330	0.430
Wean-to-estrus interval, d	5.30	5.40	5.20	5.50	0.100	0.520	0.230	0.940
Milk production⁴, kg d^{-1}	11.36^{B}	10.00^{B}	9.99^{B}	12.55^{A}	0.410	0.170	0.230	0.001
---------Litter size---------								
At 48h cross-fostering, n	13.70	13.70	13.40	13.90	0.100	0.920	0.08	0.930
At weaning, n	12.00	12.20	12.00	12.30	0.200	0.500	0.36	0.080
---------Piglet average weight---------								
At 48h cross-fostering, g	1.60	1.60	1.57	1.62	0.040	0.970	0.610	0.660
At weaning, g	6.43^{B}	6.16^{B}	5.95^{B}	6.67^{A}	0.100	0.190	0.850	0.001
Litter weight at weaning, kg	77.16^{B}	75.19^{B}	71.44^{B}	84.16^{A}	2.110	0.130	0.470	0.005
Piglet weight gain, g d^{-1}	200.00	186.00	183.00	210.00	3.000	0.340	0.680	0.003
Litter weight gain, kg d^{-1}	2.32^{B}	2.10^{B}	2.06^{B}	2.56^{A}	0.070	0.180	0.410	0.001

lactating gilts on litters performance (LSmeans).

*CON = Gilts fed with the control diet during gestation; GAA = Gilts fed with the control diet supplemented with guanidinoacetic acid (GAA), [1]Standard error of the mean. ² a.b Means followed by different lowercase letters in the lines differ (P < 0.05) from each other by Tukey test. A,B Means followed by different uppercase letters in the lines differ (P < 0.05) from each other by Tukey test, lactation treatment within gestation treatment, GT= Gestation treatments; LT= Lactation treatments; GxL= Interaction between Gestation and Lactation phases; [3]Calculated based on the equations of DOURMAD et al. (1998). [4]Daily milk production calculated considering equation of NOBLET AND ETIENNE (1989).

On d 18 of lactation, the serine levels were lower in the milk of GAA-CON gilts (P = 0.022). The levels of arginine, isoleucine, and proline tended (P < 0.10) to increase in gilts that received treatment CON-CON diets. However, all other amino acids were not influenced (P > 0.10) by treatments on d 18 of lactation (Table 6). The wean-to-oestrus interval did not differ between treatments (P > 0.10; Table 4).

DISCUSSION

Gestation phase

Gilt development and management are critical in optimizing lifetime reproductive performance. Therefore, this experiment was designed to investigate whether creatine precursor supplementation could increase or allow arginine metabolically more available to gilts. The use of guanidine acetic acid during pregnancy tends to increase the number of live piglets born without negatively impacting the litter weight. Gilts often present smaller uterine size and lower vascularization compared to multiparous sows (GAMA & JOHNSON, 1993; GUIMARÃES et al., 2014). GAA supplementation may increase available arginine in the bloodstream (MCBREAIRTY et al., 2015). The higher concentration of arginine is associated with increased angiogenesis and consequently greater uterine flow of nutrients. This increased supply throughout the gestational period may contribute to the proper distribution of nutrients to embryos and fetuses, favoring the increase in live-born piglets (CHE et al., 2013).

Table 5 - Effects of dietary guanidinoacetic acid (GAA) supplementation for gestating and lactating gilts on litters performance on the amino acid (g/16 g N) in the milk composition of first parity gilts during lactation at 7 days (LSmeans).

Parameters	-------------------Gestation phase[*]--------------------				SEM[1]	----------------P-Value[2]--------------		
	-----------------------CON GAA-----------------------							
	--------------------Lactation phase[*]--------------------							
	CON	GAA	CON	GAA		GT	LT	G x L
Dry matter (%)	20.98	19.39	22.11	19.74	0.576	0.511	0.167	0.714
---AAs content (g/16 g N)---								
Methionine	1.77^{c}	1.91^{b}	1.63^{c}	2.02^{a}	0.029	0.835	0.003	0.117
Methionine + Cysteine	1.46	1.46	1.27	1.49	0.022	0.059	0.045	0.053
Lysine	3.23^{A}	3.38^{A}	2.90^{B}	3.49^{A}	0.048	0.255	0.001	0.032
Threonine	7.06^{A}	7.21^{A}	6.34^{B}	7.86^{A}	0.113	0.897	0.009	0.026
Tryptophan	3.93^{b}	4.21^{a}	3.63^{b}	4.29^{a}	0.059	0.424	0.004	0.182
Arginine	1.30^{b}	1.37^{a}	1.12^{c}	1.33^{a}	0.020	0.038	0.013	0.173
Isoleucine	4.62^{b}	4.81^{a}	4.03^{c}	4.67^{ab}	0.064	0.018	0.009	0.118
Leucine	3.75^{b}	4.03^{a}	3.48^{b}	4.33^{a}	0.061	0.942	0.004	0.100
Valine	7.94^{b}	8.59^{a}	7.41^{b}	8.75^{a}	0.125	0.450	0.001	0.181
Histidine	4.92^{b}	5.33^{a}	4.51^{b}	5.41^{a}	0.072	0.327	0.001	0.144
Phenylalanine	2.59	2.74	2.36	2.84	0.040	0.504	0.004	0.094
Glycine	3.91	4.06	3.53	4.18	0.051	0.294	0.004	0.052
Serine	3.04^{a}	3.19^{a}	2.58^{b}	3.02^{a}	0.047	0.009	0.014	0.183
Proline	4.95^{b}	5.19^{ab}	4.48^{c}	5.26^{a}	0.078	0.266	0.013	0.143
Alanine	9.78	10.44	7.96	10.01	0.209	0.006	0.002	0.065
Asx	3.56^{b}	3.79^{a}	3.19^{c}	3.76^{a}	0.051	0.088	0.003	0.146
Glx	7.89^{b}	8.48^{a}	7.07^{c}	8.35^{a}	0.123	0.073	0.002	0.176

[*]CON = Gilts fed with the control diet during gestation; GAA = Gilts fed with the control diet supplemented with guanidinoacetic acid (GAA). [1]Standart error of the mean. [2] a.b Means followed by different lowercase letters in the lines differ ($P < 0.05$) from each other by Tukey test. A,B Means followed by different uppercase letters in the lines differ ($P < 0.05$) from each other by Tukey test, lactation treatment within gestation treatment, GT= Gestation treatments; LT= Lactation treatments; GxL= Interaction between Gestation and Lactation phases.

Due to the increased fetal and placental requirement of compounds synthesized from arginine, it is possible that arginine could be a limiting amino acid during gestation. Creatine is formed by *de novo* synthesis through methylation of GAA, formed from the amino acids glycine and arginine (HE et al., 2018). Therefore, supplementation of creatine precursors could improve the availability of arginine, which can enhance blood flow and, as a secondary result, increase the transport of creatine to the muscle tissues and improve the energy efficiency of gilts during gestation (LIU et al., 2012). In addition, the greater availability of arginine could regulate better the levels of embryonic and fetal growth and muscle development via cell signaling through targeting protein turnover, consequently increasing the size and birth weight of the litter (GAO et al., 2012; CHE et al., 2013).

Several factors can impact the efficiency of utilization and availability of dietary arginine in gilts, such as the gestational age at which the female began receiving supplementation, the parity order, the size of the litter, the environmental conditions to which the animals were subjected (PALENCIA et al., 2018), the ovulation rate, uterine capacity, and fetal survival (LI et al., 2015). Pregnant gilts have higher amino acid requirements for muscle tissue deposition (MOREIRA et al., 2020). As they are still developing, they have elevated nutritional needs for body growth, embryonic development, and adaptations for milk production. This includes increased requirements for functional nutrients, such as arginine, and higher energy demands (e.g., creatine)., which possibly explains why gilts respond well to the greater availability of amino acids during gestation allowing the animals to express better performance (HASAN et al., 2019).

Lactation phase

Gilts supplemented with GAA during gestation and lactation showed an increase in

Table 6 - Effects of dietary guanidinoacetic acid (GAA) supplementation for gestating and lactating gilts on litters performance on the amino acid (g/16 g N) in the milk composition of first parity gilts during lactation at 18 days (LSmeans).

Parameters	--------------------Gestation phase*--------------------				SEM[1]	-----------------P-Value[2]-----------------		
	-----------------------CON GAA-----------------------							
	--------------------Lactation phase*--------------------							
	CON	GAA	CON	GAA		GT	LT	Gx L
Dry matter (%)	18.85	18.66	18.15	18.57	0.203	0.345	0.784	0.456
---------------------AAs content (g/16 g N)---------------------								
Methionine	1.88	1.89	1.75	1.83	0.042	0.298	0.673	0.710
Methionine + Cysteine	1.42	1.40	1.39	1.26	0.035	0.239	0.296	0.418
Lysine	3.31	3.30	3.15	3.09	0.071	0.253	0.813	0.875
Threonine	6.72	7.32	6.69	6.88	0.181	0.551	0.330	0.604
Tryptophan	4.03	4.14	3.87	3.75	0.106	0.241	0.981	0.626
Arginine	1.34	1.37	1.21	1.17	0.043	0.084	0.959	0.688
Isoleucine	4.55	4.68	4.28	4.15	0.103	0.060	0.991	0.515
Leucine	3.94	4.07	3.78	3.83	0.103	0.385	0.712	0.855
Valine	8.13	8.29	7.93	7.78	0.168	0.359	0.988	0.680
Histidine	5.09	5.20	4.84	4.83	0.116	0.238	0.848	0.819
Phenylalanine	2.66	2.72	2.55	2.53	0.060	0.284	0.884	0.770
Glycine	3.91	4.08	3.77	3.83	0.090	0.342	0.554	0.766
Serine	2.91	3.18	2.71	2.73	0.074	0.022	0.271	0.344
Proline	4.95	5.26	4.76	4.71	0.130	0.193	0.626	0.521
Alanine	10.28	10.38	9.62	9.29	0.218	0.054	0.786	0.614
Asx	3.59	3.62	3.39	3.30	0.087	0.171	0.840	0.750
Glx	8.02	8.19	7.58	7.39	0.184	0.117	0.974	0.640

*CON = Gilts fed with the control diet during gestation; GAA = Gilts fed with the control diet supplemented with guanidinoacetic acid (GAA). [1]Standart error of the mean. [2]Means followed by different lowercase letters (a, b) in the lines differ ($P < 0.05$) from each other by the Tukey test, GT= Gestation treatments; LT= Lactation treatments; GxL= Interaction between Gestation and Lactation phases.

voluntary feed intake (+7%) during lactation. This effect on feed intake is consistent for finishing pigs fed GAA (LI and MENDOZA et al., 2018). Although, studies that demonstrated the direct mechanisms through which GAA promotes increased voluntary feed intake are scarce in the literature; however, gilts do regulate their intake by nutrient demands for productivity and body weight loss, based on the fact that GAA-GAA fed gilts produced heavier piglets (+ 491 g) and litters (+9.56 kg) and that body weight loss did not vary among treatments, the increase in voluntary feed intake could be related to the extra needs to sustain the higher production rate.

Gilts from the GAA-GAA produced more milk (+600 g d^{-1}), which can also be explained not only by the higher feed consumption but also by an increased availability of arginine and creatine (creatine phosphate) improving the energy efficiency of the mammary gland by increasing its growth and milk production capacity, contributing to piglets taking better advantage of this increased potential. Litter weight gain is correlated with milk production or nutrient concentrations in milk (NOBLET & ETIENNE, 1987; MATEO et al., 2008).

Increased piglet weight gain in gilts supplemented with GAA may be indicative of increased milk production or increased nutrient concentrations in milk.

Supplementation with GAA during gestation and lactation improved AA milk content in gilts on d 7 of lactation (i.e., lysine, threonine, methionine + cysteine, phenylalanine, glycine, alanine, tryptophan, arginine, isoleucine, leucine, valine, histidine, serine, proline, asparagine, and glutamine). It is plausible that the observed effect may be associated with the development of the mammary gland during the final trimester of gestation, which may influence the total AA concentrations in milk after the transition from colostrum to milk during the first week of lactation (KIM et al., 1999). Furthermore, MATEO et al. (2008) have reported an increase in total AA concentrations, mainly proteins, in milk on the 7th d of lactation in Arg-supplemented gilts. Those authors proposed that arginine supplementation during gestating may have stimulated mammary growth, including vascular growth, there by promoting blood flow and AA by the mammary gland to increase milk protein synthesis during this early lactation period. We can infer that the increased availability of arginine and creatine (creatine phosphate) in milk or even the improvement in mammary gland energy efficiency could have boosted mammary gland growth and increased milk production capacity, total number of piglets born alive, daily milk production, daily litter and piglet weight gain, litter and piglet weaning weights.

CONCLUSION

Dietary supplementation of guanidinoacetic acid (GAA) for gestating and lactating gilts enhances reproductive and litter performance, with effects being more pronounced when included during both phases.

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DECLARATION OF CONFLICT OF INTERESTS

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

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All methods involving animal handling were realized in accordance with the regulations approved by the Institutional Animal Welfare and Ethics/Protection committee from the Universidade Federal do Paraná (UFPR – CEUA), registered under the protocol nº 097/2017.

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