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Glutamine and vitamin A supplementation during critical periods of gestation reduces weight variability in piglets at birth

Suplementação de glutamina e vitamina A em períodos críticos da gestação de fêmeas suínas

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Abstract: Selection and genetic progress have led to the modification of the female profile, and prolificacy has become the main trait. Consequently, fetal development was affected by the increased number of piglets, resulting in higher uterine competition for nutrients and space. This study aimed to analyze the effects of glutamine and vitamin A supplementation on embryonic and fetal survival and their influence on the reproductive and productive performance of females. We selected 71 females from Large White X Landrace X Meishan X Jianjing crosses from different orders of parturition for uniform distribution between treatments, divided into two groups. Glutamine and vitamin A supplementation during periods considered critical for gestation was effective in terms of placental color, resulting in darker placentas, which could mean higher vascularization. The inclusion of glutamine and vitamin A influenced less variability in stillborn per litter and intra-litter birth weight of liveborn piglets. The treatment did not influence the mean birth weight of piglets but supplementation reduced the percentage of light piglets (≤ 1000 g) in high and low prolificacy females.

Keywords: Amino acids. Gestation. Litter. Survival.

Resumo: Com a seleção e progresso genético, o perfil das fêmeas foi modificado e a prolificidade tornou-se a principal característica. Como consequência, o desenvolvimento fetal foi afetado pelo aumento do número de leitões, o que resultou em maior competição uterina por nutrientes e espaço. O objetivo deste estudo foi analisar os efeitos da suplementação de glutamina e vitamina A na sobrevivência embrionária e fetal e sua influência no desempenho reprodutivo e produtivo de fêmeas. Foram selecionadas 71 fêmeas, oriundas dos cruzamentos Large White X Landrace X Meishan X Jianjing, de diferentes ordens de parto (OP) para a distribuição uniforme entre os tratamentos divididos em dois grupos. A suplementação de glutamina e vitamina A em períodos considerados críticos para a gestação mostrou-se efetiva neste estudo, em termos de coloração placentária, resultando em placentas mais escuras, o que poderia significar maior vascularização. A inclusão de glutamina e vitamina A influenciou em menos variabilidade em Natimortos por Leitegada (NL) e no

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Peso ao Nascer intra-leitegada dos Leitões Nascidos Vivos (PNLNV). Embora o tratamento não tenha influenciado o Peso Médio ao Nascer dos Leitões (PMNL), a suplementação reduziu a porcentagem de leitões leves (≤ 1000 g) em fêmeas de alta e baixa prolificidade.

Palavras-chave: Aminoácidos. Gestação. Leitegada. Sobrevivência.

1. Introduction

In recent years, the body composition of female pigs has been altered through genetic selection, making prolificacy and milk production the main traits for evaluation in genetic breeding programs. Consequently, other traits were also modified, such as the increase in litter weight and the number of weaned piglets/female/year, the higher number of lactations per year, and the shorter period of return to cyclicity after weaning due to a reduction in feed intake [1].

In this context, embryonic and fetal development was limited due to a decrease in blood flow to the fetus and, consequently, placental efficiency [2]. Thus, understanding the physiological changes that lead to the restriction of intrauterine growth is essential, as the adequate nutritional status of females before mating and during early, intermediate, and late gestation can be effective in increasing the uniformity of oocytes and fetuses, hence reducing the variation in embryonic development during the elongation, implantation, and placentation phases[2,3].

Glutamine is a non-essential amino acid but under specific conditions, such as critical periods of gestation and hyperprolificacy, in which endogenous production becomes insufficient to meet demand, it can become essential for metabolic regulation, increased protein synthesis, and reduced catabolism under high protein degradation conditions [4].

Similarly, vitamin A plays a crucial role in barrow reproduction in terms of increasing spermatogenesis, testicular development, and sperm production and motility [5], as well as in the reproductive traits of sows, highlighting its importance in enhancing reproduction fertility and increasing litter size [6].

However, glutamine and vitamin A supplementation in practical and commercial feed formulations for sows and their effects on reproductive performance and litter growth during sow gestation need to be further explored. This study aimed to investigate the influence of glutamine and vitamin A supplementation during critical periods of embryonic and fetal survival on reproductive and productive performance in pregnant sows.

2. Material and methods

2.1 Animals and experimental design

The procedures for handling the animals are under the ethical principles of Animal Experimentation adopted by the Animal Experimentation Ethics Committee (CEUA) of the Federal University of Uberlândia, approved under Protocol No. 90/2018. The study was conducted on a commercial pig farm located in the municipality of Patrocínio, Minas Gerais, Brazil, with latitude of 18°56′38″ S, longitude of 46°59′33″ W, and altitude of 965 m.

The experimental design was completely randomized, with two groups (control group –T1 and test group – T2), with 36 replications in the control group and 35 replications in the test group, and the sow considered the experimental unit. Seventy-one females from Large White X Landrace X Meishan X Jianjing crosses, representing different orders of parturition (OP), were selected for uniform distribution among treatments divided into two groups: T1, the control group (without supplementation), and T2, on-top supplementation with glutamine and vitamin A. Both groups included females from 1st to 8th orders of parturition, evenly distributed among the groups.

The sows were housed in a gestation shed with individual cages containing a nipple waterer and a trough feeder. They were transferred to the farrowing unit at 112 days of gestation. The gestational feed and feed management were the same as those adopted by the pig farm (Table 1). The feed used in the experiment was formulated by nutritionists from a partner company, and the formula is under commercial protection and followed animal experimentation protocols for providing supplementation to the animals.

Table 1 Ingredients and nutritional levels of the gestational diet provided to females.

Ingredient	Composition (%)
Ground wet corn grain	77.45
Soybean meal	18.50
Dicalcium phosphate	1.42
Calcitic limestone	0.80
Common salt	0.50
Gestation concentrate	1.32
Concentrate levels for use in the gestation phase (1.32 kg/ton)	
Dicalcium phosphate	25.96
Vitamins	21.05
Mycotoxin-binding agent	14.03
Lysine	10.17
Mineral supplement[1]	7.01
Choline chloride	7.01
Methionine	5.96
Threonine	4.91
Biotin	2.10
copper sulfate	1.40
Phytase	0.35

[1]*Mineral supplement composition: iron, manganese, zinc, copper, selenium, and chromium.*

The proportion used for each component of the supplement was 0.65% glutamine (Aminoscience Division – Ajinomoto do Brasil Indústria e Comércio de Alimentos Ltda, Limeira, São Paulo, Brazil) and 0.2% vitamin A. Kaolin was used as a vehicle for micronutrient fortification, ensuring homogeneous administration to all animals.

The supplements were offered considering the critical period of gestation and the dates of the gestational periods, as follows: vitamin A at 12, 27, and 35 days of gestation and glutamine at 35, 55, 70, and 100 days of gestation. All supplements were offered on the day considered a critical period of gestation, one day before and one day after this period, and three consecutive days of supplementation were allocated per period.

2.2 Measurements in females and litters

Fat thickness (FT) in females was performed in the P2 position (at the height of the last rib), approximately 6 to 7 cm from the midline on the right side, using an ultrasound (Microem®, MTU-100) pulsed at 2 MHZ. The body condition score (BCS) was evaluated using the Caliper® equipment two days before insemination and at 112 days of gestation, and three classifications were obtained: 1 (thin), 2 (ideal), and 3 (fat).

The placental visual score (PVS) was evaluated immediately after birth using the methodology mentioned in Duarte et al. [7] and then all placentas were weighed on a portable electronic hook scale (Walmur Veterinary Instruments Ltd) with an accuracy of 20 g. The placentas were collected in plastic sheets (100 cm^2) and inserted just below the vulva at the beginning of parturition to prevent the placenta from falling into the waste drainage channel.

The placentas were placed in a bucket and taken to a place with good lighting, where they were opened to observe the mummified fetuses. The visual color score was based on the color pattern from the Osava [8] methodology and classified into numbers from 1 to 6, reducing with the color of the placenta (darker placentas received a score of 1, and lighter placentas a score of 6). Placental efficiency (PE) was calculated by dividing the total litter birth weight (TLBW) by the total placental weight (TPW).

Females were subjected to estrus identification to evaluate the weaning-to-estrus interval (WEI), consisting of daily exposure to barrow from the first day after weaning until insemination of the entire herd. The characteristics measured to evaluate the performance of sows were total piglets born per litter (TPBL), stillborn piglets per litter (SPL), mummified piglets per litter (MPL), liveborn piglets per litter (LPL), total litter birth weight (TLBW), total parturitions (TP), and piglet birth weight (PBW).

Birth weight (BW) was obtained within the first 12 hours after birth, using the same portable electronic scale described previously. The effect of supplementation on gestation was evaluated considering the prolificacy of the females and was classified as low prolificacy (≤ 14 liveborn piglets) or high prolificacy (> 14 liveborn piglets) [8].

The length and circumference of liveborn, stillborn, and mummified piglets were measured to check the litter uniformity. The piglet body length was measured using a measuring tape (Circle SA®, graduated in 150 mm of 1.5 m) in the dorsal region from the insertion of the neck (occipital joint) to the base of the tail, and the circumference was measured immediately below the posterior portion of the scapula, following the dorsal and ventral regions [8].

2.2 Statistical analysis

All traits were tested for normality and homogeneity to validate the analysis of variance (ANOVA). Parametric analysis was used through ANOVA and Student's t-test was applied with a 5% significance level ($P < 0.05$), with the mean ± standard deviation. Traits that did not meet the hypothesis were analyzed using the Mann-Whitney non-parametric test as median, minimum, and maximum. Litter uniformity was analyzed using Student's t-test and variance using the F-test, with a 5% significance level ($P < 0.05$). Pearson and Spearman linear correlation analyses were performed between the variables. All analyses were performed using the software IBM® SPSS® Statistics with a significance level lower than 5% ($P < 0.05$).

3. Results

The body condition score at mating (BCSM) and the body condition score at parturition (BCSP), as well as the traits of subcutaneous fat thickness at mating (SFTM) and subcutaneous fat thickness at parturition (SFTP), did not differ between groups ($P > 0.05$) (Table 2). The means of total piglets born per litter (TPBL), liveborn piglets per litter (LPL), and stillborn piglets per litter (SPL) were 15.33 vs. 15.77, 14.61 vs. 15.20, and 0.61 vs. 0.42 between the control and supplemented groups, respectively ($P > 0.05$). No significant difference ($P > 0.05$) was observed between mummified piglets per litter (MPL) and SPL between treatments (Table 2).

Table 2 Productive and reproductive performance of females in the control and glutamine and vitamin A supplemented groups.

Trait	Control	Supplemented	P-value
TPBL	15.33 ± 3.68	15.77 ± 3.19	0.579
LPL	14.61 ± 3.47	15.20 ± 3.07	0.425
TLBW (kg)	19.15 ± 3.34	20.16 ± 3.29	0.203
TLBW + TSWL (kg)	20.04 ± 3.62	20.62 ± 3.36	0.487
TLBW + TMWL (kg)	19.17 ± 3.33	20.22 ± 3.28	0.187
TLBW + TSWL + TMWL (kg)	20.08 ± 3.62	20.68 ± 3.35	0.470
PBW (kg)	1.32 ± 0.18	1.31 ± 0.21	0.997
MPL	0.11 ± 0.11	0.14 ± 0.14	0.766
SPL	0.69 ± 0.76	0.42 ± 0.65	0.140
MP	0.30 ± 0.74	0.40 ± 0.84	0.620
PVS	3.25 ± 1.31	2.17 ± 1.22	0.0007*
TPW	4.20 ± 1.13	4.49 ± 1.16	0.535
PE (%)	4.77 ± 1.18	4.62 ± 0.79	0.918
WEI (days)	6.42 ± 5.69	5.42 ± 3.64	0.387
BCSM	2.22 ± 0.12	1.91 ± 0.12	0.081

BCSP	2.13 ± 0.12	1.91 ± 0.11	0.189
SFTM (mm)	12.69 ± 0.46	12.28 ± 0.48	0.508
SFTP (mm)	14.33 ± 0.81	12.94 ± 0.61	0.209

** Significant at 5% (P ≤ 0.05). TPBL = total piglets born per litter; LPL = liveborn piglets per litter; TLBW = total litter birth weight; TLBW + TSWL = total litter birth weight + total stillborn weight per litter; TSWL = total stillborn weight per litter; TMWL = total mummified weight per litter; TLBW + TSWL + TMWL = total litter birth weight + total stillborn weight per litter + total mummified weight per litter; PBW = piglet birth weight; NML = mummified piglets per litter; SPL = stillborn piglets per litter; MP = mummified piglets in the placenta; PVS = placental visual score; TPW = total placental weight; PE = placental efficiency; WEI = weaning-to-estrus interval; BCSM = body condition score at mating; BCSP = body condition score at parturition; SFTM = subcutaneous fat thickness at mating; SFTP = subcutaneous fat thickness at parturition. Non-parametric analysis described as mean ± standard error of the mean.*

The mean number of stillborn piglets per litter (SPL) was higher in the control group (0.61) than in the supplemented group (0.42). However, a frequency of 4% and a coefficient of variation of 123.4% of total stillborn weight per litter (TSWL) was observed in the control group and a frequency of 2% and a coefficient of variation of 152.7% was found in the supplemented group (Table 3).

Table 3 Mean, coefficient of variation (CV), and standard error of the mean (SEM) of the control and glutamine and vitamin A supplemented groups during gestation.

Trait	Mean	CV (%)	SEM[1]	P-value
Control				
ILBWLP (kg)	1.32	19.15	0.18	0.38
TPBL	19.15	17.46[B]	3.34	0.001
TLBW + TSWL (kg)	20.04	18.11	3.62	0.21
SPL	0.69	123.24[A]	0.76	0.0005
Supplemented				
ILBWLP (kg)	1.31	18.08	0.21	0.38
TPBL	20.16	16.33[A]	3.29	0.001
TLBW + TSWL (kg)	20.62	16.31	3.36	0.21
SPL	0.42	152.75[B]	0.65	0.0005

Values followed by the uppercase letters A and B in the columns differ from each other. [1]SEM = standard error of the mean; CV = coefficient of variation; ILBWLP = intra-litter birth weight of liveborn piglets; TPBL = total piglets born per litter; TLBW + TSWL = total litter birth weight + total stillborn weight per litter; SPL = stillborn piglets per litter.

Piglet birth weight (PBW) was 1.31 vs. 1.32 and the total liveborn weight (TLW) was 19.15 vs. 20.16 in the control and supplemented groups, showing no significant difference (P > 0.05). The coefficient of variation (CV) of the total liveborn weight (TWL) showed a significant difference between the control and supplemented groups (P = 0.0001). Mummified piglets in the placenta (MP), placental efficiency (PE), and placental weight (PW) did not differ between groups (P > 0.05). The placental visual score (PVS) showed a highly significant difference (P = 0.0007) between the control and supplemented groups (Table 2).

No effect of treatment or interaction between treatments and prolificacy class was observed on the body weight of piglets ≤ 1000 g (P > 0.05). The low prolificacy class showed a higher percentage of piglets ≤ 1000 g in the control group compared to the supplemented group, with values of 8.93 vs. 6.94%, respectively. The same percentage pattern was also observed in the high prolificacy class, in which the control group had 28% of piglets ≤ 1000 g and the supplemented group had 25%. The group that received the supplement in both classes had a lower percentage of piglets weighing less than or equal to 1000 g (Table 4).

Table 4 Weight and percentage of light piglets (≤ 1000 g) according to litter size classification and groups.

Trait	Control	Supplemented
Low prolificacy (≤ 14 liveborn piglets)		
Number of females	19	13
MBW (kg)	1.45±0.02	1.52 ± 0.02
piglets ≤ 1000 g (%)	8.93	6.94
High prolificacy (> 14 liveborn piglets)		
Number of females	17	22
MBW (kg)	1.22 ± 0.01	1.24 ± 0.01
piglets ≤ 1000 g (%)	28	25

Control = group without supplementation; Supplemented = group with Vitamin A supplementation on gestation days D11 to D13 and D26 to D28, vitamin A + glutamine on gestation days D34 to D36, and glutamine on gestation days D54 to D56, D69 to 71, and D99 to D101; MBW = mean birth weight of piglets.

4. Discussion

Although the body condition score at mating (BCSM) and body condition score at parturition (BCSP), as well as subcutaneous fat thickness at mating (SFTM) and subcutaneous fat thickness at parturition (SFTP) did not differ between groups (P > 0.05), females presented higher SFTP and loss of BCSM in the control group. Females accumulated more fat when compared to the supplemented group, with fatter females resulting in worse body structure, which may be related to the lack of amino acids, such as glutamine.

Females use other amino acids to synthesize specific compounds they require, thus resulting in a loss of BCS, which may reflect on the total piglets born per litter (TPBL), as observed in this study, with values of 19.15 vs. 20.16 in the control and supplemented groups, respectively. Moreover, females at the end of gestation are in a catabolic state due to limited protein intake and increased protein requirements to support the growth of fetal tissues and mammary glands.

The results of this study corroborate those of Zhu et al. [9], who used glutamine supplementation for 85 days of gestation until parturition and found no differences in the total number of born, liveborn, and stillborn piglets. In this study, the total piglets born per litter (TPBL) did not differ between groups, and the lower variability is associated with reduced mortality and better performance until weaning, with no significance (P > 0.05).

On the other hand, the intra-litter birth weight of liveborn piglets (ILBWLP) was lower in the supplemented group.

Placental visual score (PVS) showed a highly significant difference ($P < 0.001$), indicating that the supplemented group had a darker-colored placenta, which could mean higher vascularization when compared to the control group. Arginine acts as a precursor of nitric oxide, which plays an important role in the dilation of maternal systemic circulation and regulation of uterine and placental blood flow [10].

Therefore, glutamine supplementation during periods that included mid and late gestation is assumed to be essential for a lower percentage of piglets with light color and in low and high prolificacy classes, as supplemented females had a lower placenta visual score (PVS), that is, darker placentas, which may mean higher vascularity. Low birth weight piglets have inadequate colostrum intake [11,12], which results in low acquisition of passive immunity and poor nutritional status, increasing the incidence of deaths or reduced performance of piglets in later stages [12].

In general, studies with feed supplementation, especially amino acids during gestation, provide an improvement in the productive and reproductive performance of pigs, leading to the recognition of the amino acids arginine and glutamine as essential for gestation [13].

5. Conclusion

Glutamine and vitamin A supplementation in periods considered critical for gestation was effective in this study in terms of placental color, resulting in darker placentas, which could mean higher vascularization. Glutamine and vitamin A inclusion influenced less variability in stillborn piglets per litter (SPL) and intra-litter birth weight of liveborn piglets (ILBWLP). Although the treatment did not influence the mean birth weight of piglets (MBW), supplementation reduced the percentage of light piglets (≤ 1000 g) in high and low prolificacy females.

Declaration of conflict of interest

The authors have no competing interests.

Authors' contributions

Conceptualization: A.L. Bernardes. *Data curation*: G.S. Vieira and J.S. Soares. *Formal analysis*: J.S. Soares and R.C. Antunes. *Methodology*: J.S. Soares and S.S. Rabelo. *Project administration*: A.L. Eugênio. *Software*: J.S. Soares. *Supervision*: R.C. Antunes. *Validation*: R.C. Antunes. *Writing (original draft)*: A.C.R. Cunha. *Writing (review & editing)*: A.C.R. Cunha.

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