N-carbamoylglutamate supplementation in late-gestation dairy cows is associated with increased newborn weight and improved nutrient supply in the placentome

Fengfei Gu

Zhejiang University

Luyi Jiang

Zhejiang University

Linyu Xie

Zhejiang University

Diming Wang

Zhejiang University

Jian-Xin Liu (✉ liujx@zju.edu.cn)

Zhejiang University Institute of Dairy Science <https://orcid.org/0000-0002-5812-5186>

Research

Keywords: N-carbamoylglutamate, dairy cows, newborn weight, placentome, metabolomics

Posted Date: July 27th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-47833/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

N-carbamoylglutamate (NCG), a structural analog of N-acetylglutamate that is essential for endogenous arginine synthesis, has unique advantages, including low rumen degradability, low cost, and a lack of negative effects on intestinal absorption of dietary histidine and lysine. Information is limited on the effect of dietary NCG on the reproduction performance of the late-gestation dairy cows. The objective of this study was to investigate whether supplementation of NCG to cows during late gestation alters uteroplacental tissue nutrient transporters, calf metabolism and newborn weight.

Results

Thirty multiparous Chinese Holstein cows were used in an experiment with a randomized complete block design. During the last 28 d of pregnancy, cows were fed a diet without (CON) or with NCG (20 g/d/cow). The mRNA expression of genes related to arginine, glucose, and fatty acid transporters, angiogenesis factors and the mTOR pathway in the placentome were analyzed. Blood samples of calves were collected for analysis of plasma parameters, amino acids, and metabolites. The newborn weight ($P < 0.01$) and plasma arginine concentrations ($P = 0.07$) were higher in NCG calves than in CON calves. The mRNA expression of genes involved in glucose transport, angiogenesis and the mTOR pathway in the placenta was upregulated ($P < 0.01$) in NCG-supplemented cows. In addition, 17 significantly different metabolites identified were mainly involved in arginine and proline metabolism; alanine, aspartate and glutamate metabolism and the citrate cycle.

Conclusions

These results suggest that the increased expression of angiogenesis factors and glucose transporters and the activation of mTOR signaling pathways in the uteroplacenta that occurs through changes in gene transcription under NCG supplementation may be contribute to an increased nutrient supply and improved amino acid metabolism and urea cycling in the fetus.

Background

Calf birth weight is strongly associated with calf health, growth, and future productivity. In dairy cows, the final trimester of gestation is characterized by marked fetal growth, and abundant nutrients are required to ensure adequate fetal growth [1]. Placental development and functions play important roles in the growth and development of the fetus. Placental angiogenesis supports the required blood flow to the fetal side; thus, increased angiogenesis could improve fetal growth [2]. The expression and activity of specific transporters in the placenta influence the transport of nutrients from the maternal to fetal circulation in nonruminant animals [3]. Batistel et al. [4] reported that increased mRNA abundance of

genes encoding amino acid (AA), glucose and fatty acid transporters in the placenta could increase the birth weight of dairy calves. In addition to having the indispensable function of delivering nutrients essential for fetal growth, placental tissue (as do other mammalian tissues) has an array of nutrient-sensing signaling pathways, such as the mammalian target of rapamycin (mTOR) complex [5]. The mTOR pathway can alter the activity of placental nutrient transporters, such as AA transporters [6].

Arginine (Arg) is a functional AA with various physiological functions [7]. Many studies have shown that Arg regulates placental function and has great impacts on fetal survival, growth and development [8, 9]. Dietary supplementation of Arg products can prevent fetal growth restriction in humans [10] and rats [11]. Supplementation of rumen-protected Arg has been shown to improve placental development in ewes [12] and promote intestinal absorption of AA by regulating the mTOR signaling pathway [13]. However, the high rumen degradation and high price of Arg limit its utilization in dairy cows. Alternatively, N-carbamoylglutamate (NCG), a structural analog of N-acetylglutamate that is essential for endogenous Arg synthesis [14], has unique advantages, including low degradability in the rumen, low cost, and a lack of negative effects on intestinal absorption of dietary histidine and lysine [15]. Cai et al. [16] reported that NCG has great potential to improve pregnancy outcomes in sows. In gilts, maternal NCG supply during early pregnancy improves embryonic survival and development through modulation of endometrial proteomes [17, 18]. In our previous study, we found that supplemental NCG in transitional dairy cows could increase the supply of Arg and improve the health of dairy cows [19].

Taken together, the objective of the present study was to investigate whether supplementation of NCG to dairy cows during late pregnancy affects fetal growth and newborn weight by altering uteroplacental tissue nutrient transporters and calf metabolism.

Materials And Methods

Animals and experimental design

The experimental procedures used in this study were approved by the Animal Care Committee of Zhejiang University (Hangzhou, China) and conducted in accordance with the university's guidelines for animal research. The design of the experiment is described in our previous study [19]. In brief, thirty multiparous Chinese Holstein dairy cows at wk 4 before parturition with similar BW (657 kg, SD = 58) were assigned to 15 blocks according to parity and the 305-day milk yield (8692 kg, SD = 607) of the previous lactation. The first week (wk 4 before parturition) was used as the adaptive week. The dairy cows were then randomly allocated into two groups and fed basal diet alone (CON) or supplemented with 20 g/d NCG (NCG) (Beijing Animore Sci. & Tech. Co., Ltd., Beijing, China). The chemical compositions of the diets are listed in Supplementary Table S1. Throughout the trial period, cows were housed in a barn with individual tie stalls and had free access to water. NCG was added once per day at 1400 h by scattering it on the total mixed ration for individual cows. Calves were weighed soon after parturition and before colostrum consumption.

Blood sample collection and analyses

Fourteen and fifteen blood samples from each newborn calf was collected from the jugular vein before colostrum consumption in the CON and NCG group respectively. Then these blood samples centrifuged at $3,000 \times g$ for 15 min at 4°C for collection of plasma and then frozen at -80°C until subsequent analysis. A subsample of each plasma sample was used to analyze biochemical variables, including glucose, total protein, blood urea nitrogen, non-esterified fatty acids, β -hydroxybutyrate, cholesterol, triglyceride, total bilirubin, albumin, and globulin using an AutoAnalyzer 7020 instrument (Hitachi High-technologies Corporation, Tokyo, Japan) with colorimetric commercial kits (Ningbo Medical System Biotechnology Co., Ltd.). The plasma AA compositions were determined as described elsewhere [19]. Six plasma subsamples in each group were collected based on the following criteria: parities of the dairy cows, number of days from the data, the cows began receiving NCG to the calving day, and calf sex (female) to analyze the metabolome profiles with GC-MS.

Placentome collection and analyses

The placentome samples were collected referring to the methods reported by Batistel et al. [4]. Briefly, after natural delivery (within 2 h), the placenta was rinsed with physiologic saline, and then, 4-6 placentomes from the central area of the placenta were dissected, rinsed with physiologic saline until clear and then stored at -80°C for later analysis. The criteria of sample selection were based on the following: parities and body conditions of the dairy cows, number of days from the data the cows began receiving NCG to the calving day, time when the placenta was released after parturition, and calf sex. According to these criteria, six placentas were selected from each group for further analysis.

Total RNA from the placentome was extracted with TRIzol reagent according to the manufacturer's instructions (Aidlab Inc.; Code: RN03). The concentration and purity of the total RNA were measured using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). The total RNA of the placentome was reverse transcribed to cDNA using the PrimeScript 1st Strand cDNA Synthesis Kit (TOYOBO, Osaka, Japan; Code: FSQ-101). Quantitative real-time PCR (qRT-PCR) was performed with the 2 × SYBR® Premix Ex Taq kit (Aidlab Inc., Beijing, China; Code No. PC5902) and the Applied Biosystems 7500 system (Foster City, CA). The PCR conditions were set as follows: 1 cycle at 95°C for 2 min, 40 cycles of 95°C for 15 s and 60°C for 34 s, followed by a melting curve program (from 60 to 95°C).

Twenty-three genes of interest were selected based on their key roles in placental functions: (1) genes encoding Arg transporters (*SLC6A14*, *SLC7A1*, *SLC7A6*, *SLC7A7*, *SLC7A9*); (2) genes encoding glucose transporters (*SLC2A1*, *SLC2A3*, *SLC2A4*); (3) genes encoding fatty acid transporters (*SLC27A1*, *SLC27A2*, *SLC27A3*); (4) genes associated with angiogenesis (*VEGFA*, *NOS3*, *GUCY1B3*, *HIF1A*), and (5) genes involved in the mTOR pathway (*AKT1*, *mTOR*, *RPS6KB1*, *EIF4BP1*, *EIF4EBP2*, *EEF1A1*, *ELF2*, *IRS1*). In addition, two references genes (*GAPDH* and *ACTB*) were included based on published recommendations [4]. Some of the primers targeting these genes were selected based on previous studies, and others were designed using National Center for Biotechnology Information Nucleotide database (<http://www.ncbi.nlm.nih.gov/nuccore/>), with *Bos taurus* as the species (Supplementary Table S2). The

specificity of the primers was validated by primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The relative changes at the mRNA level for each individual gene were analyzed using the $2^{-\Delta\Delta CT}$ method, where $\Delta CT = CT_{\text{target mRNA}} - CT_{\text{housekeeping mRNA}}$ (where CT = cycle threshold).

Metabolomics analysis

Fifty microliters of sample was transferred into a 2 mL tube, and 200 μL prechilled extraction mixture (methanol) and 5 μL internal standard (L-2-chlorophenylalanine, 1 mg/mL stock) were added. The mixture was then vortex mixed for 30 s. The samples were then ultrasonicated for 10 min in ice water and centrifuged at 4°C for 15 min at $16,065 \times g$. To prepare the quality control (QC) sample, 20 μL of each sample was collected and pooled together. After evaporation in a vacuum concentrator, 30 μL of methoxyamination hydrochloride (20 mg/mL in pyridine) was added. The mixture was then incubated at 80°C for 30 min and then derivatized by 40 μL of BSTFA reagent (1% TMCS, v/v) at 70°C for 1.5 h. After gradually cooling the samples to room temperature, 5 μL of FAMES (in chloroform) was added to the QC sample. All samples were then analyzed by a gas chromatograph coupled with a time-of-flight mass spectrometer (GC-TOF-MS).

Raw data processing, including peak extraction, baseline adjustment, deconvolution, alignment and integration [20], was performed with Chroma TOF (V 4.3x, LECO) software, and the LECO-Fiehn Rtx5 database was used for metabolite identification by matching the mass spectrum and retention index. Finally, the peaks detected in fewer than half the QC samples or $RSD \geq 30\%$ in the QC samples was removed [21].

The pattern recognition multivariate analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), were performed with SIMCA 14.1 software package (V14.1, Sartorius Stedim Data Analytics AB, Umea, Sweden) with log transformation and the unit variance scaling conversion mode. The significantly different metabolites (SDMs) were defined based on the variable importance for the projection (VIP) > 1.0 and P value < 0.05 [22]. The SDMs were further identified and validated by searching the online Kyoto Encyclopedia of Genes and Genomes (KEGG) and Bovine Metabolome Database (BMDB). Metaboanalyst 3.0 (<http://www.metaboanalyst.ca/>) was employed to identify relevant pathways. The *Bos taurus* (cow) pathway library was applied in this procedure.

Statistical analysis

Blood parameters and mRNA expression levels of the placentome were analyzed using SAS software (version 9.0) with covariance type AR(1) and the MIXED model procedure, with treatment included as a fixed effect and block and cow as random effects. The means were separated using the PDIFF option of the LSMEANS procedure. The experimental results were reported as least squares means. Significance was declared at $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered indicative of a trend.

Results

Body weight, plasma parameters and aa composition of newborn calves

The body weight of newborn calves in the two groups are shown in Fig. 1. The body weight of NCG calves was significantly higher than that of CON calves (37.3 kg vs. 32.2 kg; $P < 0.01$). The plasma parameters of calves before colostrum consumption are presented in Table 1. Compared with that in the control group, the blood urea nitrogen concentration tended to be lower in the NCG group ($P = 0.09$); no differences in the other plasma parameters were observed between the two groups. Plasma Arg concentration tended to be higher in the NCG group ($P = 0.07$), and no difference in the concentrations of other AAs was observed between the two groups (Table 2).

Gene expression of the placentome

The mRNA expression of genes associated with Arg, glucose and fatty acid transporters, angiogenesis factors, and the mTOR signal pathway in the placentome are presented in Fig. 2. Supplementation of NCG during late gestation resulted in increased mRNA expression of *SLC2A3* ($P < 0.01$) and *NOS3* ($P < 0.01$) in the placentome of dairy cows. The mRNA levels of *AKT1* ($P = 0.09$), *mTOR* ($P = 0.10$) and *EIF4BP1* ($P = 0.07$) tended to be higher and mRNA those of *EIF4EBP2* ($P = 0.01$) and *ELF2* ($P = 0.01$) were significantly higher in the NCG group than in the CON group.

Metabolite profiling using GC-TOF/MS

A total of 303 effective peaks were obtained after data processing, of which 145 were quantified at the relative level and 158 were labeled “analyte” or “unknown” based on analysis via the LECO-Fiehn Rtx5 database. The results of the multivariate analysis of metabolic profile differences between the CON and NCG calves are shown in Fig. 3. No clear distinction was found between the two groups according to the PCA score plot (Fig. 3A). In the PCA model, the R^2X value was 0.577. The OPLS-DA score plot showed separated clusters between the CON and NCG samples, (Fig. 3B). Two evaluation parameters for the classification from the OPLS-DA model were calculated: $R^2Y = 0.971$ and $Q^2 = 0.365$. The results of permutations are presented in Fig. 3C, with intercept $R^2 = (0, 0.86)$ and $Q^2 = (0, -0.42)$, indicating that the OPLS-DA model was sufficiently robust for downstream analysis.

Identification of significantly different metabolites and pathways

Seventeen SDMs and 7 significantly different unidentified peaks were found between the CON and NCG groups (Fig. 4A). Among these SDMs, citrulline [Log₂-fold change (FC) = 0.49] was more abundant in the NCG group, while 9 SDMs were enriched in the CON group: three metabolites of AA metabolism, i.e., urea [Log₂ (FC) = -0.76], glutamine 3 [Log₂ (FC) = -0.63] and N-acetyltryptophan 2 [Log₂ (FC) = -0.71]; two organic acids, i.e., succinic acid [Log₂ (FC) = -0.87] and cyclohexyl sulfamic acid 1 [Log₂ (FC) = -0.70]; and four alcohols, i.e., cholestane-3,5,6-triol [Log₂ (FC) = -1.06], phytol [Log₂ (FC) = -0.66], 1-hexadecanol [Log₂ (FC) = -0.71] and citraconic acid 4 [Log₂ (FC) = -1.24]. Detailed information on the SDMs is provided in Supplementary Table S3.

Seventeen SDM-enriched metabolic pathways were identified and are illustrated in a metabolome view map (Fig. 4B). These SDMs were mainly involved in the pathways Arginine and proline metabolism ($P = 0.001$, impact value = 0.027); Alanine, aspartate and glutamate metabolism ($P = 0.007$, impact value = 0.127); and Citrate cycle (TCA cycle, $P = 0.109$, impact value = 0.01). Detailed information on the identified metabolic pathways is provided in Supplementary Table S4.

Discussion

In our previous study, dietary NCG supplementation increased the AA supply and improved the health status of transition dairy cows [19]. The nutrient supply and maternal health are closely related to fetal growth and development [23]. Arginine is one of the most versatile AAs and plays multiple physiological functions in animals [24, 25]. The placenta is the channel through which the fetus obtains nutrients and oxygen from the mother, and it plays important roles in the growth and development of the fetus. Maternal nutrient availability and placental transport efficiency are major intrauterine factors influencing fetal development [26]. Adequate vasculogenesis and angiogenesis of the placenta are also important for providing adequate maternal nutrients and blood flow to the fetus. As a precursor of nitric oxide (NO), Arg could enhance placental angiogenesis [16, 27]. In the current study, *NOS3*, an important angiogenesis factor [28], was expressed in the placenta at higher levels in the dairy cows receiving NCG than in the control cows, indicating that vasculogenesis and angiogenesis of the placenta may have been enhanced by NCG supplementation. This effect of NCG might be attributable to an increase in NO concentration [19] and enhances nutrients and oxygen delivery to the fetus, promoting fetal growth and development.

Angiogenesis and blood flow are the transport belts for nutrients, and fetal access to maternal nutrients requires the involvement of nutrient transporters. In the current study, the plasma Arg concentration was higher in the newborn calves of cows receiving NCG than in the newborns of CON cows. In addition to with the Arg transporters in the placentome, these results suggest that the higher Arg content in the newborn NCG-group calves may be attributable to higher Arg contents in their mothers and not to the expression of placental transporters. However, the expression of glucose transporter (*SLC2A3*) was enhanced in the NCG group relative to the CON group. As the main energy, glucose is required for the fetal growth and metabolism. Bell [29] and Gao et al. [30] reported that fetal gluconeogenesis is marginal in cows, and the fetus is mainly dependent on glucose from maternal circulation. The transports of glucose and other polyols, such as fructose, galactose, mannose, and maltose in the placenta, occur by facilitated diffusion along a concentration gradient through members of the GLUT family, such as GLUT1 (*SLC2A1*) and GLUT3 (*SLC2A3*) [31]. These transporters have a heterogeneous distribution across the placental membrane. For example, GLUT1 is mainly located in the basal membrane, whereas GLUT3 is predominant in the microvillus membrane; the sequential action of both transporters is involved in glucose transport to the fetal circulation [32]. Therefore, the increased expression of *SLC2A3* observed in NCG cows in this study indicates that the changes in glucose transporters occurred mainly in the microvillus membrane, which is rich in capillaries and may be related to placental angiogenesis. In addition, the AA concentration and supply increased with NCG supplementation in dairy cows [19], contributing to enhanced glucose transport into the placenta [4, 33].

In addition to its nutritional transporter roles, which affect the acquisition of maternal nutrients by the fetus, the placenta tissue has nutrient-sensing signaling pathways, such as the mTOR complex [5]. This complex is a serine/threonine protein kinase that may regulate cell growth and proliferation via activation of the ribosomal protein S6 kinase to phosphorylate ribosomal protein S6 and consequently regulate protein synthesis and other processes [34, 35]. Nutrient levels, especially those of AAs, represent major upstream regulators of mTOR. The activation of mTOR in the placenta determines fetal growth, and placental mTOR constitutes a mechanistic link between maternal nutrient availability and fetal growth [6]. Batistel et al. [4] reported that supplemental methionine during late gestation increases feed intake and body weight in newborn calves, possibly via the involvement of mTOR signaling in the placenta. Zhang et al. [13] reported that Arg can help mitigate the negative effect of intrauterine growth restriction on nutrient absorption in neonatal lambs by regulating the mTOR signaling pathway. Glucose and individual AAs, such as leucine and Arg, can independently regulate mTOR signaling [36]. Therefore, the enhanced mRNA abundance of genes associated with mTOR signaling observed in the NCG group in the current study may be attributed to the increased Arg concentration in dairy cows receiving NCG supplementation. This phenomenon may contribute to the delivery of maternal nutrients to the fetus, leading to improved fetal growth.

Metabolomic analysis of the plasma of calves indicated that maternal NCG supplementation increased fetal plasma citrulline levels and improved AA metabolism and the citrate cycle. These findings are consistent with the higher Arg concentration in newborn calves of NCG cows and our previous finding in transition dairy cows that Arg levels increased under NCG supplementation [19]. Arginine plays a key role in ureagenesis as a precursor of citrulline during the urea cycle [37]. Therefore, these results suggest that the fetuses of NCG-supplemented cows may have obtained more maternal Arg than the fetuses of CON cows and thus experienced improved citrulline synthesis and urea cycling, as reflected in their lower blood levels of urea nitrogen.

Conclusions

In conclusion, supplementation of NCG in dairy cows during late gestation increases newborn body weight. This effect of NCG supplementation may be mediated by improved uteroplacental angiogenesis, enhanced glucose transport and activated mTOR signaling in the placenta. These alterations improve the amino acid metabolism and nitrogen utilization efficiency of the fetus.

Abbreviations

AA, amino acid; Arg, arginine; BHB, β -hydroxybutyrate; BUN, blood urea nitrogen; CON, control; mTOR, mammalian target of rapamycin complex; NCG, N-carbamoylglutamate; NEFA, non-esterified fatty acids; NO, nitric oxide; OPLS-DA, orthogonal partial least squares discriminant analysis; PCA, principal component analysis; SDM, significantly different metabolites; SEM, standard error of the mean.

Declarations

Ethics approval and consent to participate

All animal experiments and procedures were approved by the Animal Use and Care Committee of Zhejiang University (Hangzhou, China).

Consent for publication

Not applicable.

Availability of data and materials

All data measured or analyzed during this work are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was financially supported by grants from the China-USA Intergovernmental Collaborative Project in S & T Innovation under the National Key R & D Program (Grant No. 2018YFE0111700) and the China Agriculture Research System (Grant No. CARS-36).

Author contributions

FFG, DMW, and JXL designed the research; FFG, LYJ, and LYX conducted the research; FFG analyzed the data; and FFG and DMW wrote the paper. JXL has primary responsibility for the final content. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the staff of the Hangjiang Dairy Farm (Hangzhou, China) for milking and caring for the animals. We also acknowledge the members of the Institute of Dairy Science Zhejiang University (Hangzhou, China) for their assistance with sampling and sample analysis.

References

1. NRC. Nutrient requirements of dairy cattle. 7th rev. ed. Washington, DC: National Academy Press. 2001.

2. Liu XD, Wu X, Yin YL, Liu YQ, Geng MM, Yang HS, et al. Effects of dietary L-arginine or N-carbamylglutamate supplementation during late gestation of sows on the miR-15b/16, miR-221/222, VEGFA and eNOS expression in umbilical vein. *Amino Acids*. 2012;42:2111–19.
3. Jones HN, Powell TL, Jansson T. Regulation of placental nutrient transport - a review. *Placenta*. 2007;28:763–74.
4. Batistel F, Alharthi AS, Wang L, Parys C, Pan YX, Cardoso FC, et al. Placentome nutrient transporters and mammalian target of rapamycin signaling proteins are altered by the methionine supply during late gestation in dairy cows and are associated with newborn birth weight. *J Nutr*. 2017;147:1640–47.
5. Jansson N, Rosario FJ, Gaccioli F, Lager S, Jones HN, Roos S, et al. Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *J Clin Endocrinol Metab*. 2013;98:105–13.
6. Roos S, Powell TL, Jansson T. Placental mTOR links maternal nutrient availability to fetal growth. *Biochem Soc Trans*. 2009;37:295–8.
7. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Rhoads JM, et al. Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*. 2009;37:153–68.
8. Santana PD, Silva TV, da Costa NN, da Silva BB, Carter TF, Cordeiro MS, et al. Supplementation of bovine embryo culture medium with L-arginine improves embryo quality via nitric oxide production. *Mol Reprod Dev*. 2014;81:918–27.
9. Wu GY, Bazer FW, Satterfield MC, Li XL, Wang XQ, Johnson GA, et al. Impacts of arginine nutrition on embryonic and fetal development in mammals. *Amino Acids*. 2013;45:241–56.
10. Xiao XM, Li LP. L-Arginine treatment for asymmetric fetal growth restriction. *Int J Gynaecol Obstet*. 2005;88:15–8.
11. Vosatka RJ, Hassoun PM, Harvey-Wilkes KB. Dietary L-arginine prevents fetal growth restriction in rats. *Am J Obstet Gynecol*. 2005;178:242–6.
12. Zhang H, Sun L, Wang Z, Deng M, Nie H, Zhang G, et al. N-carbamylglutamate and L-arginine improved maternal and placental development in underfed ewes. *Reprod*. 2016;151:623–35.
13. Zhang H, Peng A, Yu Y, Guo S, Wang M, Coleman DN, et al. N-carbamylglutamate and L-arginine promote intestinal absorption of amino acids by regulating the mTOR signaling pathway and amino acid and peptide transporters in suckling lambs with intrauterine growth restriction. *J Nutr*. 2019;149:923–32.
14. Gessler P, Buchal P, Schwenk HU, Wermuth B. Favorable long-term outcome after immediate treatment of neonatal hyperammonemia due to N-acetylglutamate synthase deficiency. *Eur J Pediatr*. 2010;169:197–9.
15. Chacher B, Wang DM, Liu HY, Liu JX. Degradation of L-arginine and N-carbamoylglutamate and their effect on rumen fermentation in vitro. *Ital J Anim Sci*. 2012;11:4693–6.
16. Cai S, Zhu J, Zeng X, Ye Q, Ye C, Mao X, et al. Maternal N-carbamylglutamate supply during early pregnancy enhanced pregnancy outcomes in sows through modulations of targeted genes and

- metabolism pathways. *J Agric Food Chem*. 2018;66:5845–52.
17. Zhang B, Che LQ, Lin Y, Zhuo Y, Fang ZF, Xu SY, et al. Effect of dietary N-carbamylglutamate levels on reproductive performance of gilts. *Reprod Domest Anim*. 2014;49:740–5.
 18. Zhu JL, Zeng XF, Peng Q, Zeng SM, Zhao HY, Shen HX, et al. Maternal N-carbamylglutamate supplementation during early pregnancy enhances embryonic survival and development through modulation of the endometrial proteome in gilts. *J Nutr*. 2015;145:2212–20.
 19. Gu FF, Miao C, Jiang LY, Wang DM, Liu HY, Liu JX. Dietary supplementation with N-carbamoylglutamate initiated during the prepartum stage improves the lactation performance of postpartum dairy cows. *Anim Nutr*. 2020; (in press).
 20. Kind T, Wohlgemuth G, Lee DY, Lu y, Palazoglu M, Shahbaz S, et al. FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. *Anal Chem*. 2009;81:10038–48.
 21. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. The Human Serum Metabolome (HUSERMET) Consortium. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc*. 2011;6:1060–83.
 22. Sun HZ, Wang DM, Wang B, Wang JK, Liu HY, Guan LL, et al. Metabolomics of four biofluids from dairy cows: potential biomarkers for milk production and quality. *J Proteome Res*. 2015;14:1287–98.
 23. Tao S, Monteiro AP, Thompson IM, Hayen MJ, Dahl GE. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. *J Dairy Sci*. 2012;95:7128–36.
 24. Morris SMJ. Recent advances in arginine metabolism: roles and regulation of the arginases. *Br J Pharmacol*. 2009;157:922–30.
 25. Tan BE, Li XG, Wu GY, Yin YL. Dynamic changes in blood flow and oxygen consumption in the portal-drained viscera of growing pigs receiving acute administration of L-arginine. *Amino Acids*. 2012;43:2481–89.
 26. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. *Int J Mol Sci*. 2014;15:16153–85.
 27. Raghavan SAV, Dikshit M. Vascular regulation by the L-arginine metabolites, nitric oxide and agmatine. *Pharmacol Res*. 2004;49:397–414.
 28. Wu X, Yin YL, Liu YQ, Liu XD, Liu ZD, Li TJ, et al. Effect of dietary arginine and N-carbamoylglutamate supplementation reproduction and gene expression of eNOS, VEGFA and PlGF1 in s placenta in late pregnancy of sows. *Anim Reprod Sci*. 2012;132:187–92.
 29. Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J Anim Sci*. 1995;73:2804–19.
 30. Gao H, Wu G, Spencer TE, Johnson GA, Bazer FW. Select nutrients in the ovine uterine lumen. II. Glucose transporters in the uterus and periimplantation conceptuses. *Biol Reprod*. 2009;80:94–104.

31. Baumann MU, Deborde S, Illsley NP. Placental glucose transfer and fetal growth. *Endocrine*. 2002;19:13–22.
32. Wooding FBP, Fowden AL, Bell AW, Ehrhardt RA, Limesand SW, Hay WW. Localisation of glucose transport in the ruminant placenta: implications for sequential use of transporter isoforms. *Placenta*. 2005;26:626–40.
33. Brown LD, Green AS, Limesand SW, Rozance PJ. Maternal amino acid supplementation for intrauterine growth restriction. *Front Biosci*. 2011;3:428–44.
34. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev*. 2004;18:1926–45.
35. Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell*. 2006;124:471–84.
36. González IM, Martin PM, Burdsal C, Sloan JL, Mager S, Harris T, et al. Leucine and arginine regulate trophoblast motility through mTOR-dependent and independent pathways in the preimplantation mouse embryo. *Dev Biol*. 2012;361:286–300.
37. Meijer AJ, Lof C, Ramos IC, Verhoeven AJ. Control of ureogenesis. *Eur J Biochem*. 1985;148:189–96.

Tables

Table 1. Blood parameters of newborn calves from the late gestation dairy cows that supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d

Item ¹	CON	NCG	SEM	P-value
Glucose, mmol/L	3.31	2.51	0.38	0.20
Total protein, g/L	46.5	45.0	1.17	0.41
BUN, mmol/L	3.22	2.81	0.14	0.09
NEFA, μ mol/L	650	490	231	0.64
BHBA, μ mol/L	37.4	38.8	4.38	0.83
Cholesterol, mmol/L	0.64	0.64	0.08	0.98
Triglyceride, mmol/L	0.16	0.13	0.03	0.64
Total bilirubin, μ mol/L	6.00	8.20	2.90	0.62
Creatinine, μ mol/L	203	239	31.0	0.46
Albumin (A), g/L	23.8	23.7	0.36	0.94
Globulin (G), g/L	22.7	21.2	1.05	0.37
A/G ratio	1.02	1.13	0.05	0.17

¹ NEFA, Non-esterified fatty acid; BHBA, β -hydroxybutyrate; BUN, blood urea nitrogen.

Table 2. Plasma amino acid composition of newborn calves that from the late gestation dairy cows that supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d

Amino acid (umol/L)	CON	NCG	SEM	<i>P</i> -value
Essential amino acids				
Arginine	62.9	89.9	8.94	0.07
Threonine	534	651	119	0.51
Valine	172	200	30.1	0.54
Methionine	18.9	29.6	6.82	0.30
Isoleucine	39.7	56.4	14.5	0.44
Leucine	65.9	79.7	18.4	0.61
Phenylalanine	160	179	10.5	0.24
Lysine	50.4	82.1	22.3	0.35
Histidine	100	118	15.1	0.45
Total	1,204	1,485	235	0.42
None-essential amino acids				
Alanine	523	737	191	0.45
Cysteine	12.6	17.6	3.63	0.37
Tryptophan	28.6	26.9	3.74	0.75
Glutamic acid	122	159	23.9	0.31
Glycine	732	745	51.1	0.87
Asparagine	42.5	47.6	4.81	0.48
Serine	118	127	8.45	0.49
Proline	157	275	98.1	0.42
Total	1,736	2,135	248	0.37
Total amino acid	2,940	3,620	585	0.44

Figures

Figure 1

Birth weight of newborn calves from the later gestation dairy cows supplemented with 0 (CON) or 20 g/d of N-carbamoylglutamate (NCG). $P < 0.01$: significant difference between two groups. Bars indicate the standard error of the mean.

Figure 2

The mRNA expression of transporters and mTOR signally pathway in placentome of dairy cows without (CON) or with N-carbamoylglutamate (NCG) at 20g/d. * $P < 0.05$; ** $P < 0.01$. Bars indicate the standard error of the mean.

Figure 3

The PCA score map, OPLS-DA score plot and corresponding validation plot of OPLS-DA derived from the metabolite profiles of experimental calves. The red circle represents CON-calves whose mother dairy cows had no supplementation with N-carbamoylglutamate (NCG), while the blue box represents NCG-calves whose mothers were supplemented with NCG at 20g/d. PCA = principal component analysis. OPLS-DA = orthogonal partial least squares discriminant analysis.

Figure 4

Volcano plot of serum metabolites (A) and metabolome view map of the significantly different metabolites (B) identified in calves from the dairy cows supplemented with 0 (CON) or 20 g/d of N-carbamoylglutamate (NCG). In the A, the x-axis represents $\log_2$ (FC) value, and the y-axis means $-\log_{10}$ (P value). The red dot indicates the significantly different metabolite (SDM) that are more abundant in the NCG group, while the blue dot represents the SDM with higher concentration in the CON cows. The dot size represents the variable importance in the projection (VIP) value. FC=fold change, mean value of peak area obtained from the NCG group/mean value of peak area obtained from the CON group. In the B, the x-axis represents the pathway impact, and the y-axis represents the pathway enrichment. Larger sizes and darker colors represent higher pathway impact values and higher pathway enrichment, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupportingInformation.docx](#)