Postprint: Effects of Late Gestation Nutritional Restriction on Gastrointestinal Glucose Transporter-Related Gene Expression in Ewes

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Abstract

This study aimed to investigate the effects of nutritional restriction during late pregnancy on the expression of glucose transporter-related genes in the gastrointestinal tract of ewes. Twenty Xiangdong black goats with synchronized pregnancies were selected and randomly divided into two groups: a control group (ad libitum feeding) and a restricted feeding group (40% feed intake restriction), with 10 goats per group. The preliminary period lasted 15 days (gestation days 81-95), and the formal experimental period lasted 39 days (gestation days 96-135). At the end of the formal experimental period, the goats were slaughtered, and mucosal samples from the rumen, duodenum, jejunum, ileum, and cecum were collected. Real-time quantitative PCR (qPCR) technology was used to detect the expression levels of sodium-glucose cotransporter 1 (SGLT1), sodium-glucose cotransporter 3 (SGLT3), facilitative glucose transporter 2 (GLUT2), and facilitative glucose transporter 5 (GLUT5) genes. The results showed that, compared with the control group, SGLT1 gene expression in the rumen was significantly decreased ($P<0.05$) and tended to decrease in the jejunum and ileum ($0.05 P<0.10$); GLUT5 gene expression in the cecum was significantly decreased ($P<0.05$); while the expression of other glucose transporter genes in the gastrointestinal tract showed no significant differences between the restricted feeding and control groups ($P>0.05$). It can be concluded that nutritional restriction during late pregnancy in ewes exerted varying degrees of influence on the expression of glucose transporter genes in the gastrointestinal tract, thereby causing alterations in glucose transport within the ewe' s body.

Full Text

Effects of Nutritional Restriction during Late Gestation on Gene Expressions of Glucose Transporters in the Gastrointestinal Tract of Ewes

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Abstract: This study investigated the effects of nutritional restriction during late gestation on gene expression of glucose transporters in the gastrointestinal tract of ewes. Twenty pregnant Xiangdong black goats were selected and randomly assigned to two groups: a control group (ad libitum feeding) and a restricted group (40% feed intake restriction), with 10 ewes per group. The pre-trial period lasted 15 days (gestation days 81–95), and the formal trial period lasted 39 days (gestation days 96–135). At the end of the trial, the ewes were slaughtered to collect mucosal samples from the rumen, duodenum, jejunum, ileum, and cecum. Real-time quantitative PCR was used to detect the expression levels of sodium-glucose cotransporter 1 (SGLT1), sodium-glucose cotransporter 3 (SGLT3), facilitated glucose transporter 2 (GLUT2), and facilitated glucose transporter 5 (GLUT5) genes. The results showed that compared with the control group, SGLT1 gene expression in the rumen of the restricted group was significantly decreased ($P<0.05$), with a decreasing trend observed in the jejunum and ileum ($0.05 P<0.10$). GLUT5 gene expression was significantly reduced in the cecum ($P<0.05$), while no significant differences were observed for other glucose transporter genes in the gastrointestinal tract between the two groups ($P>0.05$). These findings indicate that nutritional restriction during late gestation in ewes differentially affects the expression of glucose transporter genes in the gastrointestinal tract, thereby altering glucose transport in the maternal body.

Keywords: late gestation; nutritional restriction; goat; glucose transporter

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Carbohydrates are indispensable nutrients for animal growth, metabolism, and production performance. Ruminants possess a complex stomach physiology distinct from monogastric animals, with carbohydrate utilization occurring through two primary pathways: first, fermentation by rumen microorganisms to produce volatile fatty acids (VFAs) that are absorbed by rumen epithelial cells; second, degradation in the intestine to monosaccharides such as glucose that are ab-

sorbed by intestinal epithelial cells [1]. Glucose serves crucial physiological functions in ruminants as the main energy substrate and a precursor for lactose synthesis. Therefore, glucose absorption and transport in the gastrointestinal tract are essential for maintaining ruminant health and ensuring production performance. Previous studies have demonstrated that glucose or starch infusion into the abomasum can rapidly increase glucose transporter activity [2]. Prolonged nutritional restriction leads to adaptive adjustments in glucose transporter expression, enabling animals to cope with low-nutrient states [3]. However, existing research cannot fully explain the changes in glucose transport in ruminants under nutritional restriction conditions. Moreover, China's grassland animal husbandry is severely affected by seasonal variations, with grazing livestock experiencing weight losses of up to 30% from the withered grass period to the green-up period [4]. During the withered grass period, livestock face severe nutritional limitations that inevitably cause nutritional imbalances and reduced production performance [5]. Metabolic disorders are the primary cause of decreased production performance in ruminants, with particularly severe impacts on ewes during late gestation under low-nutrient conditions. While numerous production practices exist, research on the effects of nutrition on gastrointestinal glucose transporters remains scarce.

Therefore, this study investigated changes in glucose transporter gene expression in the gastrointestinal tract of Liuyang black goats under nutritional restriction during late gestation, aiming to provide a theoretical basis for scientific feeding and management of pregnant goats.

1. Materials and Methods

The feeding trial was conducted from January to June 2015 at the Liuyang Black Goat Nutrition and Metabolism Technology Innovation Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

1.1 Experimental Animals

Experimental animals were provided by the Innovation Experimental Base. Sixty Xiangdong black goat ewes of similar age [(2.0±0.3) years], second parity, good body condition, and similar body weight [(25.0±1.0) kg] with synchronized estrus were selected. Estrus synchronization, artificial insemination, and B-ultrasound examination were employed to ensure synchronized pregnancy. Twenty ewes carrying single fetuses were selected from the pregnant ewes as experimental subjects.

1.2 Experimental Design

A single-factor randomized design was adopted. On day 81 of gestation, the 20 selected ewes were randomly allocated to two groups: a control group (ad libitum feeding, CG) and a restricted group (40% feed intake restriction, RG), with 10 ewes per group. The pre-trial period lasted 15 days (gestation days 81–

95) for environmental adaptation, during which daily feed intake was recorded. The formal trial period lasted 39 days (gestation days 96–135).

1.3 Feeding Management

During gestation days 0–80, all experimental ewes were collectively housed and allowed to graze freely. From gestation days 81–95, the selected ewes were housed in individually ventilated pens with suitable temperature and humidity for a 15-day adaptation period. Ewes were fed a diet with a concentrate-to-forage ratio of 60:40. Diet composition and nutrient levels are shown in Table 1 , with the forage being fresh Miscanthus from local Liuyang mountains, chopped before feeding. Ewes were fed at 08:30 and 17:00 daily with free access to water. Feed allowance was gradually increased with gestational age, adjusted every 7 days based on the ad libitum intake of the control group. However, the restricted group received 60% of their intake during the first week of the adaptation period.

1.4 Sample Collection and Index Determination

At the end of the formal trial period, ewes were slaughtered for sample collection. Six ewes were randomly selected from each group and slaughtered via carotid artery exsanguination. After respiration ceased, the animals were dissected for sampling. Tissue samples were collected from five gastrointestinal regions: rumen, duodenum, jejunum, ileum, and cecum. Samples were rinsed with physiological saline, and mucosa was scraped using glass slides, quickly wrapped in sterilized aluminum foil, and preserved in liquid nitrogen. After completion of the slaughter trial, samples were stored at -80 °C for subsequent analysis.

Gene expression levels of glucose transporters were detected, including two families: (1) Sodium-glucose cotransporters (SGLTs), comprising sodium/glucose cotransporter member 1 (SGLT1) and sodium/glucose cotransporter member 3 (SGLT3); and (2) facilitated glucose transporters (GLUTs), comprising facilitated glucose transporter member 2 (GLUT2) and facilitated glucose transporter member 5 (GLUT5).

1.5 Real-time Quantitative PCR

1.5.1 Main Instruments Conventional surgical instruments (scalpel, surgical scissors, forceps, etc.), autoclave (Tomy SX-500, Japan), clean bench (SW-CJ-IFD, Antai Air Technology Co., Ltd.), ultra-low temperature centrifuge (Hitachi CR22G II, Japan), central water purification system (ELAG LAB Water CENTRA200, UK), ultra-micro UV spectrophotometer (NanoDrop ND2000, USA), real-time PCR instrument (Roche Light Cycler 480 II, Switzerland), gel imaging system (Ultro-Violet, UK), electrophoresis apparatus (ECP3000, Beijing Liuyi Instrument Factory), and electrophoresis tank (DYCP-34A, Beijing Liuyi Instrument Factory).

1.5.2 Main Reagents 0.9% physiological saline, chloroform, isopropanol, diethylpyrocarbonate (DEPC) water, 75% ethanol (prepared with DEPC water), RNAiso Plus (9109, TaKaRa, Japan), PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A, TaKaRa, Japan), Premix Taq™ (Ex Taq™ version 2.0 plus dye) (RR902A, TaKaRa, Japan), SYBR® Premix Ex Taq™ II (RR802A, TaKaRa, Japan), ethidium bromide (EB), 50×TAE (ST716, Beyotime Biotechnology), and agarose (GelPilot LE Agarose D40724, QIAGEN, Germany).

1.5.3 Total RNA Extraction and Reverse Transcription Total RNA was extracted using RNAiso Plus reagent, and purity and concentration were determined using an ultra-micro UV spectrophotometer. Total RNA with an OD260 nm/OD280 nm ratio of 1.8–2.2 was considered pure. After 1% agarose gel electrophoresis, RNA quality was assessed based on a 2:1 grayscale ratio of 28S rRNA to 18S rRNA. The PrimeScript RT reagent Kit was used for reverse transcription of total RNA to synthesize cDNA.

1.5.4 Real-time Quantitative PCR Primers for glucose transporter genes (SGLT1, SGLT3, GLUT2, GLUT5) were designed using Primer Premier 5.0 software based on goat gene sequences from GenBank, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene. Primer specificity was analyzed using the NCBI Primer-BLAST tool. All primers were synthesized by Shanghai Sangon Biotech Co., Ltd., with sequences listed in Table 2 .

The 10 L real-time PCR reaction system included: 5.0 L SYBR® Premix Ex Taq™ II, 0.2 L forward primer (10 mol/L), 0.2 L reverse primer (10 mol/L), 3.6 L RNase-free dH2O, and 1.0 L cDNA. The reaction program consisted of denaturation at 95 °C for 5 s and extension at 60 °C for 30 s for 40 cycles, followed by a melting program of 95 °C for 15 s and 60 °C for 15 s.

1.6 Data Processing and Analysis

Real-time PCR data were analyzed using the 2^(-ΔΔCt) method [6] for relative quantification of gene expression. All data were analyzed using SPSS 20.0 software with independent samples t-test to verify significant differences, where $P<0.05$ indicated significant differences between groups and $0.05 P<0.10$ indicated a trend. Results are expressed as mean ± standard error.

2. Results

2.1 Effects of Nutritional Restriction during Late Gestation on SGLTs Gene Expression in the Gastrointestinal Tract of Ewes

As shown in Table 3 , compared with the control group, SGLT1 gene expression in the cecum was significantly decreased under nutritional restriction during late gestation ($P<0.05$). Although no significant differences were observed in the rumen, duodenum, jejunum, and ileum ($P>0.05$), SGLT1 expression showed

decreasing trends in the jejunum and ileum (0.05 P<0.10), decreasing by 54.5% and 63.7% compared with the control group, respectively.

Table 4 shows that SGLT3 gene expression in different gastrointestinal regions was not significantly affected by nutritional restriction during late gestation (P>0.05).

2.2 Effects of Nutritional Restriction during Late Gestation on GLUTs Gene Expression in the Gastrointestinal Tract of Ewes

As shown in Table 5 , GLUT2 gene expression was not significantly affected by nutritional restriction in the rumen, duodenum, jejunum, ileum, or cecum (P>0.05), although a slight increase was observed in the duodenum (41.6% higher than the control group).

Table 6 reveals that GLUT5 gene expression was significantly decreased in the cecum under nutritional restriction during late gestation (P<0.05), while no significant changes were observed in the rumen, duodenum, jejunum, or ileum (P>0.05).

3. Discussion

3.1 SGLTs Family

Glucose transporters are classified into two families based on transport mechanisms: the SGLTs family and the GLUTs family [7]. SGLTs are primarily expressed on the apical membrane of cells and transport glucose and galactose [8], whereas GLUTs are mostly expressed on the basolateral membrane and transport fructose, maintaining glucose homeostasis through negative feedback regulation when glucose concentrations are low [9]. Studies indicate that glucose derived from sucrose or starch primarily enters intestinal epithelial cells via SGLT1 [10-11], and SGLT1 expression is regulated by feed intake and circadian rhythms at both transcriptional and translational levels [12]. In newborn mammals before weaning, SGLT1 expression is strong in the intestine, facilitating high hexose uptake capacity, but decreases significantly after weaning, reducing hexose absorption [7]. Bauer et al. [13] found that -glucose infusion significantly increased SGLT1 activity in the jejunum of cattle and sheep, thereby enhancing glucose absorption efficiency. Other studies have shown that feed restriction in piglets reduces SGLT1 and GLUT2 gene expression [14]. Our results demonstrate that SGLT1 expression tended to decrease in the jejunum and ileum and was significantly reduced in the cecum under nutritional restriction during late gestation. This may be attributed to decreased glucose concentration in the gastrointestinal tract caused by nutritional restriction, leading to reduced SGLT1 expression to meet normal physiological demands while avoiding unnecessary energy loss and nutrient waste.

Human medical research indicates that SGLT3 can induce Na+ current changes but does not participate in glucose transport [15]. In animal models such as mice,

SGLT3 comprises two subtypes (mSGLT3a and mSGLT3b), with mSGLT3a function remaining unclear, while mSGLT3b has been confirmed to induce Na+ current changes without transporting glucose [16]. Therefore, SGLT3-encoded proteins may function as sensors rather than transporters [12]. Our study found that SGLT3 expression increased by 25.6% in the duodenum under nutritional restriction, but not in posterior intestinal segments. This may be because the duodenum is the first intestinal segment receiving chyme from the abomasum, allowing SGLT3, as a sensor, to immediately detect changes in gastrointestinal glucose content, thereby increasing its expression. This suggests that monitoring SGLT3 expression enables more precise sensing of glucose content in chyme, regulating glucose transporter expression and metabolism in posterior intestinal segments to prevent unnecessary energy expenditure and nutrient loss.

3.2 GLUTs Family

Studies have shown that SGLT1 and GLUT2 function not only as glucose transporters but also as glucose-sensing receptors that stimulate gastrointestinal hormone secretion [17-20]. Artificial sweeteners can increase glucose absorption efficiency by upregulating GLUT2 expression when stimulating brush border membranes [20]. In our study, GLUT2 expression was unaffected by nutritional restriction, with the highest expression observed in the duodenum. Similar to our findings, Yoshikawa et al. [11] reported that the anterior gastrointestinal tract (rumen and duodenum) is the primary site of GLUT2 function. Additionally, when SGLT1 activity on the brush border membrane reaches saturation, GLUT2 can transiently translocate to the apical membrane to conserve energy and alleviate the reduction in Na+ gradient caused by SGLT1 activity changes [12]. Our results suggest that nutritional restriction may reduce glucose concentration in posterior intestinal segments (jejunum, ileum, and cecum), preventing SGLT1 activity from reaching saturation and consequently reducing GLUT2 expression in these regions.

In cell models such as intestinal epithelial L-cell lines, GLUT5 expression is high under fructose stimulation [21], suggesting that fructose absorption and transport in the intestine may occur via GLUT5. This has been confirmed in animal models, as GLUT5-knockout mice cannot absorb fructose, indicating that fructose transport is GLUT5-dependent [22]. Given that elevated GLUT5 expression indicates enhanced fructose transport capacity, our finding of highest GLUT5 expression in the duodenum suggests that this is the primary site of fructose transport. Furthermore, our results reveal that despite nutritional restriction, GLUT5 expression remained high in the anterior intestine, while significantly decreasing in the cecum. In summary, nutritional restriction during late gestation differentially affects glucose transporter expression in the gastrointestinal tract, allowing us to optimize dietary formulations based on production requirements to improve performance.

References

[1] Zhang X. Effects of different dietary nutrient levels on metabolism and intestinal nutrient sensing in cashmere goats[D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2014.
[2] Zhang H, Liu Q, Wang C, et al. Effects of 2-methylbutyrate on enzyme activities and glucose transporter gene expression in calf small intestine[J]. Scientia Agricultura Sinica, 2016, 49(5): 979-987.
[3] Xu Q, Tian KX. Research progress on glucose metabolism in ruminants[J]. Feed Review, 2009(8): 17-20.
[4] Liu X G, Li D B, Hou X Z, et al. Effects of nutritional restriction and compensation on small intestinal mucosal growth and development in lambs[J]. Scientia Agricultura Sinica, 2011, 44(17): 3613-3621.
[5] Wu D Q, He Z X, Tang S X, et al. Effects of nutritional restriction during late gestation on meat quality and related gene expression in lambs[J]. Life Science Research, 2013, 17(2): 151-155.
[6] Schmittgen T D, Livak K J. Analyzing real-time PCR data by the comparative CT method[J]. Nature Protocols, 2008, 3(6): 1101-1108.
[7] He W Y, Su L H, Song Z, et al. Research progress on intestinal glucose transporters[J]. Hunan Agricultural Sciences, 2013(7): 8-11.
[8] Kellett G L, Brot-Laroche E, Mace O J, et al. Sugar absorption in the intestine: the role of GLUT2[J]. Annual Review of Nutrition, 2008, 28: 35-54.
[9] Kalsi K K, Baker E O, Fraser O, et al. Glucose homeostasis across human airway epithelial cell monolayers: role of diffusion, transport and metabolism[J]. Pflügers Archiv: European Journal of Physiology, 2008, 457(5): 1061-1070.
[10] Wright E M, Loo D D F, Hirayama B A. Biology of human sodium glucose transporters[J]. Physiological Reviews, 2011, 91(2): 733-794.
[11] Yoshikawa T, Inoue R, Matsumoto M, et al. Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract[J]. Histochemistry and Cell Biology, 2011, 135(2): 183-194.
[12] Tolhurst G, Reimann F, Gribble F M. Intestinal sensing of nutrients[J]. Handbook of Experimental Pharmacology, 2012, 209: 309-335.
[13] Bauer M L, Harmon D L, McLeod K R, et al. Adaptation to small intestinal starch assimilation and glucose transport in ruminants[J]. Journal of Animal Science, 1995, 73(6): 1828-1838.
[14] Liu J, Liu Z, Gao L, et al. Nutrient-intake-level-dependent regulation of intestinal development in newborn intrauterine growth-restricted piglets via glucagon-like peptide-2[J]. Animal, 2016, 10(10): 1645-1654.
[15] Díez-Sampedro A, Hirayama B A, Osswald C, et al. A glucose sensor hiding in a family of transporters[J]. Proceedings of the National Academy of Sciences of the United States of America, 2003, 100(20): 11753-11758.
[16] Díez-Sampedro A, Barcelona S. Sugar binding residue affects apparent Na+ affinity and transport stoichiometry in mouse sodium/glucose cotransporter type 3B[J]. Journal of Biological Chemistry, 2011, 286(10): 7975-7982.
[17] Kellett G L. Comment on: Gorboulev et al. Na+-D-glucose cotransporter

SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes 2012;61:187-196[J]. Diabetes, 2012, 61(6): e4.
[18] Moriya R, Shirakura T, Ito J, et al. Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice[J]. American Journal of Physiology: Endocrinology and Metabolism, 2009, 297(6): E1358-E1365.
[19] Cani P D, Holst J J, Drucker D J, et al. GLUT2 and the incretin receptors are involved in glucose-induced incretin secretion[J]. Molecular and Cellular Endocrinology, 2007, 276(1/2): 18-23.
[20] Mace O J, Schindler M, Patel S. The regulation of K- and L-cell activity by GLUT2 and the calcium-sensing receptor in the small intestine[J]. The Journal of Physiology, 2012, 590(12): 2917-2936.
[21] Reimann F, Habib A M, Tolhurst G, et al. Glucose sensing in L cells: a primary cell study[J]. Cell Metabolism, 2009, 8(6): 532-539.
[22] Barone S, Singh A K, Zuo J, et al. Slc2a5 (GLUT5) is essential for the absorption of fructose in the intestine and generation of fructose-induced hypertension[J]. The Journal of Biological Chemistry, 2009, 284: 5056-5066.

Note: Figure translations are in progress. See original paper for figures.