Effects of Dietary Nutrient Levels on Relative mRNA Expression of Small Intestinal Sensing Factors, Blood Physicochemical Indices, and Hormone Content in Cashmere Goats (Post-print)

Authors: Zhang Xia, Sun Haizhou, Sangdan, Zhao Cunfa, Li Shengli, Glamorous City, Ling Shuli, Shandan, Ren Xiaoping

Date: 2017-10-11T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of dietary N-carbamylglutamate (NCG) supplementation and duodenal glucose infusion on mRNA relative expression of intestinal nutrient sensing factors, blood physicochemical indices, and hormone concentrations in cashmere goats under conditions of appropriately reduced dietary nitrogen levels. Twenty-seven healthy Inner Mongolian white cashmere wethers fitted with permanent rumen and duodenal fistulas were randomly allocated to 9 groups (n=3 per group) based on similar age and body weight. Three dietary treatments were designed: low nitrogen [crude protein (CP) 10.5%], low nitrogen + NCG (0.20 g/d), and high nitrogen (CP 13.5%); goats in each dietary treatment received three levels of duodenal glucose infusion: 0, 20, and 40 g/d. After the feeding trial (15-day preliminary period and 15-day formal period), goats were slaughtered to collect jejunum and duodenum tissues. The mRNA relative expression of nutrient sensing factors was determined by real-time quantitative PCR, while blood physicochemical indices and serum and jejunal hormone concentrations were measured. The results showed that: 1) Under basal dietary conditions (without glucose infusion), decreasing dietary nitrogen level reduced the mRNA relative expression of sodium-glucose cotransporter 1 (SGLT1) in jejunum and duodenum, plasma urea nitrogen and glucose concentrations, serum citrulline and insulin concentrations, and serum and jejunal glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), and glucose-dependent insulinotropic peptide (GIP) concentrations, but increased the mRNA relative expression of solute carrier family 7 member 9 (SLC7A9) and solute carrier family 7 member 1 (SLC7A1) in jejunum and duodenum. 2) With increased

duodenal glucose infusion, decreasing dietary nitrogen level tended to increase the mRNA relative expression of SGLT1, taste receptor type 1 member 1 (T1R1), taste receptor type 1 member 2 (T1R2), and taste receptor type 1 member 3 (T1R3). 3) Under low nitrogen diet conditions with 20 g/d glucose infusion, additional NCG supplementation alleviated the decrease in mRNA relative expression of SGLT1 in jejunum and duodenum, plasma urea nitrogen and glucose concentrations, serum citrulline concentration, and serum and jejunal GLP-1, GLP-2, and GIP concentrations induced by reduced dietary nitrogen level. These results suggest that appropriately reducing dietary nitrogen level, supplemented with NCG and increased rumen-protected glucose (duodenal infusion of 20 g/d), exerts promoting effects on metabolism and intestinal nutrient sensing in cashmere goats.

Full Text

Effects of Dietary Nutrient Level on mRNA Relative Expression Levels of Intestinal Nutrient Sensing Factors, Blood Physiochemical Indexes and Hormone Contents in Cashmere Goats

ZHANG Xia[1,2], SUN Haizhou[2]*, SANG Dan[2], ZHAO Cunfa[2], LI Shengli[2], YAN Cheng[2], LING Shuli[2], SHAN Dan[2], REN Xiaoping[2]

[1]Inner Mongolia Fuyuan Farming Co., Ltd., Hohhot 010070, China
[2]Institute of Animal Nutrition and Feed, Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences, Hohhot 010031, China

Abstract: The current experiment aimed to investigate the effects of N-carbamylglutamic acid (NCG) supplementation and duodenal glucose infusion on mRNA relative expression levels of intestinal nutrient sensing factors, blood physiochemical indexes, and hormone contents in cashmere goats under conditions of reduced dietary nitrogen level. Twenty-seven healthy Inner Mongolia cashmere wethers fitted with permanent rumen and duodenal fistulas were selected and randomly divided into nine groups (n=3) according to similar age and body weight. Three dietary treatments were designed: low nitrogen [crude protein (CP) 10.5%], low nitrogen + NCG (0.20 g/d), and high nitrogen (CP 13.5%). Goats in each treatment received duodenal glucose infusion at three levels: 0, 20, and 40 g/d. Following the feeding trial (15-day pre-period and 15-day formal period), goats were slaughtered to collect jejunal and duodenal tissues. The mRNA relative expression levels of nutrient sensing factors were determined by real-time quantitative PCR, while blood physiochemical indexes and hormone contents in serum and jejunum were measured. The results showed: 1) Under basal dietary conditions (without glucose infusion), as dietary nitrogen level decreased, the mRNA relative expression levels of sodium-glucose cotransporter 1 (SGLT1) in jejunum and duodenum, plasma urea nitrogen and glucose contents, serum citrulline and insulin contents, and serum and jejunal

contents of glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2), and glucose-dependent insulinotropic peptide (GIP) decreased, whereas the mRNA relative expression levels of solute carrier family 7 member 9 (SLC7A9) and solute carrier family 7 member 1 (SLC7A1) increased. 2) After increasing appropriate rumen-bypass glucose, with decreasing dietary nitrogen level, the mRNA relative expression levels of SGLT1, taste 1 receptor member 1 (T1R1), taste 1 receptor member 2 (T1R2), and taste 1 receptor member 3 (T1R3) tended to increase. 3) Under low nitrogen dietary conditions with 20 g/d glucose infusion, additional NCG supplementation could alleviate the decrease in mRNA relative expression level of SGLT1 in jejunum and duodenum, plasma urea nitrogen and glucose contents, serum citrulline content, and serum and jejunal contents of GLP-1, GLP-2, and GIP induced by reduced dietary nitrogen level. These results suggest that appropriately reducing dietary nitrogen level, supplementing NCG, and increasing rumen-bypass glucose (duodenal infusion of 20 g/d) can promote metabolism and intestinal nutrient sensing in cashmere goats.

Key words: goat; intestinal nutrient sensing factor; glucose; N-carbamylglutamic acid

---

The intestine accounts for 25% of whole-body oxygen consumption and serves as the primary site for food digestion and nutrient absorption. It functions as an independent integrated system that regulates gastrointestinal functions, earning it the designation of "second brain." Simultaneously, the intestine acts as a chemosensory interface responsible for transmitting information generated from the gastrointestinal lumen environment to the brain and other body parts [1]. As early as 1964, McIntyre et al. [2] discovered that oral glucose administration was more effective than intravenous injection in increasing plasma insulin content, thereby confirming the positive role of the gastrointestinal tract in sensing and transmitting nutrient signals. Lam et al. [3] proposed the concept of "gastrointestinal chemosensing," noting that its direct research significance lies in improving animal feed intake, enhancing nutrient digestion and absorption, strengthening intestinal barrier function, promoting gut health, and thereby comprehensively improving livestock productivity [4].

Dietary carbohydrates are primarily absorbed and utilized as glucose in the small intestine. Glucose transport from the small intestine to blood occurs through two main pathways: active transport and facilitated diffusion [5]. The active transport pathway involves sodium-glucose cotransporter 1 (SGLT1) located on the brush border membrane of intestinal epithelial cells, which couples glucose or galactose with water and transports two sodium molecules from the intestinal lumen into blood through the electrochemical gradient maintained by basolateral Na+/K+-ATPase [6]. The facilitated diffusion pathway is accomplished by glucose transporter 2 (GLUT2), a low-affinity glucose transporter located on both apical and basolateral membranes [7].

Furthermore, taste sensors originally found in the oral cavity were discovered and first reported in the intestine in 2002 [8]. Taste sensors comprise two types of G protein-coupled receptors (GPCRs): taste receptor type 1 (T1R) and taste receptor type 2 (T2R) [9-10]. The sweet taste sensor, formed as a heterodimer of T1R2 and T1R3 from the T1R family, primarily detects monosaccharides (such as glucose, galactose, and fructose) in the intestine [11]. Meanwhile, the T1R1/T1R3 heterodimer senses umami (savory) taste and can recognize L-amino acids available in the intestine due to its ability to detect "umami" flavor produced by glutamate [12]. The T2R family represents bitter taste sensors and consists of at least 30 GPCR members. Sweet, umami, and bitter receptors are linked through -gustducin, a G protein expressed in A, K, and L cells [9-10], which secrete related hormones [glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2), cholecystokinin (CCK), etc.] to promote nutrient absorption.

Currently, the concept of low-nitrogen feeding has gradually penetrated livestock and poultry production. However, research progress in this field has been relatively slow for ruminants due to their complex physiological structure. During the advancement of research on dietary carbohydrate regulation techniques and functional amino acids from single to multi-dimensional perspectives, fundamental studies on the interaction between dietary protein (amino acids) and glucose and their effects on metabolism have become particularly important. Therefore, this experiment was conducted to determine the effects of diets with different nitrogen and glucose levels supplemented with N-carbamylglutamic acid (NCG) on blood physiochemical indexes and related hormone contents in cashmere goats, and to measure mRNA relative expression levels of intestinal nutrient sensing factors through real-time quantitative PCR. The objective was to investigate the effects of NCG supplementation and appropriate glucose infusion on glucose absorption in the small intestine of cashmere goats under low nitrogen levels, thereby enabling rational diet formulation, reducing resource waste, and lowering feed costs.

Materials and Methods

1.1.1 Experimental Design

Twenty-seven healthy Inner Mongolia cashmere wethers with permanent rumen fistulas and duodenal cannulas, weighing (50.07±5.97) kg, were selected and randomly divided into nine groups (n=3) according to similar age and body weight. Three dietary treatments were designed: low nitrogen [crude protein (CP) 10.5%], low nitrogen + NCG (0.20 g/d [13-14]), and high nitrogen (CP 13.5%), designated as LN, LN+NCG, and HN, respectively. Goats in each treatment received duodenal glucose infusion at three levels: 0, 20, and 40 g/d, designated as 0 g/d G, 20 g/d G, and 40 g/d G. Experimental procedures followed the protocols described in our previous studies [15-18].

Experimental diets were formulated according to NRC (2007) [19], with composition and nutrient levels shown in Table 1 . NCG was purchased from Beijing Union Biotechnology Co., Ltd. and subjected to rumen-protected processing by Beijing Feed Science and Technology Co., Ltd. (rumen protection rate 92%).

Table 1 Composition and nutrient levels of experimental diets (air-dry basis)

Items	Ingredients/%	Low nitrogen LN	Low nitrogen+NCG	High nitrogen HN
Ingredients	Chinese wildrye			
	Alfalfa			
	Soybean meal			
	Corn			
	Wheat bran			
	Premix[1]			
	Total			
Nutrient levels[2]	ME/(MJ/kg)			
	DM/%			
	CP/%	10.5	10.5	13.5
	Ca/%			
	P/%			
	NDF/%			
	NFC/%			
	NFC:NDF			

[1] One kilogram of premix contains the following: Fe (FeSO ·7H O) 170 g, Cu (CuSO ·5H O) 70 g, Mn (MnSO ·5H O) 290 g, Zn (ZnSO ·7H O) 240 g, Co (CoCl ·6H O) 510 mg, KI 200 mg, NaSeO 130 mg, VA 620,000 IU, VD 324,000 IU, VE 540 IU, VK 150 mg, VB 0.9 mg, VB 450 mg, calcium pantothenate 750 mg, folic acid 15 mg.

[2] Nutrient levels were calculated values, with methods referenced to AFRC (1993, British) and Feedstuffs Ingredient Analysis Table, 2007 ed. (USA) [20-21]; NFC = 1 - NDF - CP - EE - ash.

Experimental goats were individually housed in feeding cages and fed equal amounts of forage and concentrate at 07:00 and 16:00 daily.

1.2.1 Serum and Plasma Sample Collection

Blood samples were continuously collected from experimental goats on days 8-10 of the formal period. At 08:00 each day, 20 mL of blood was collected from the jugular vein. Ten milliliters was slowly injected into centrifuge tubes coated

with heparin (750 IU) (kept in an ice box), and plasma samples were separated by centrifugation at 2,000×g for 15 min at 4°C within 30 min. Prepared samples were stored at -25°C for subsequent measurement of plasma glucose and urea nitrogen contents.

The remaining 10 mL of blood was placed in tubes, centrifuged at 4,500×g for 10 min to prepare serum samples. Serum was transferred to centrifuge tubes, sealed, and stored at -20°C for measurement of serum GLP-1, GLP-2, GIP, CCK, insulin, and citrulline contents. Results represent the average of three days.

1.2.2 Intestinal Tissue Sample Collection for Hormone Measurement

After the feeding trial, one representative goat from each group was slaughtered. Approximately 10 cm of the mid-jejunum was collected, rinsed with warm physiological saline, and rapidly divided into ~2 cm segments that were placed in cryotubes and stored at -80°C. For hormone content measurement, jejunal tissue samples were homogenized to determine GLP-1, GLP-2, GIP, and CCK contents.

1.2.3 Sample Collection for Real-time Quantitative PCR

Following the method for qPCR sample collection, the entire small intestine was removed by opening the abdominal cavity. After cutting the mesentery, portions of duodenum and anterior jejunum were opened, rinsed with warm physiological saline, rapidly aliquoted, and stored in liquid nitrogen for subsequent analysis. The mRNA relative expression levels of basic amino acid transporters [solute carrier family 7 member 9 (SLC7A9), solute carrier family 7 member 1 (SLC7A1)], intestinal glucose transporters (SGLT1, GLUT2), and nutrient sensing factors (T1R1, T1R2, T1R3) were determined in jejunum and duodenum of goats from different groups.

1.3.2 Plasma Glucose Content

Plasma glucose content was measured using an automatic biochemical analyzer (Beckman-Coulter, USA).

1.3.3 Serum Insulin and Citrulline, Serum and Jejunal GLP-1, GLP-2, GIP, and CCK Contents

Serum GLP-1, GLP-2, GIP, CCK, and insulin were determined by ELISA using kits provided by Beijing Xin Fangcheng Biotechnology Co., Ltd. For measurement of GLP-1, GLP-2, GIP, and CCK in jejunal tissue samples, jejunal tissue was homogenized and measured using the same method as for serum. Serum citrulline content was also determined by ELISA using kits from Jianglai Chemical Technology (Shanghai) Co., Ltd.

1.4 Experimental Instruments and Reagents

Instruments included: microplate reader (Labsystems Multiskan MS, Finland), plate washer (Thermo Labsystems AC8, Finland), centrifuge (micro high-speed centrifuge, TG16W), incubator (water-jacketed constant temperature incubator, GNP-9080), high-speed refrigerated centrifuge (Eppendorf 5417, Germany), micro UV spectrophotometer (Biodropsis, Beijing Wuzhou Oriental Science & Technology Development Co., Ltd.), low-temperature centrifuge (Eppendorf 5810, Germany), PCR instrument (Illumina Eco, USA), gel imaging system, real-time PCR instrument (ABI MP3005, USA), and ultra-low temperature freezer (Thermo Forma, USA).

Reverse transcription PCR, real-time PCR kits, and Ex-Taq premix were produced by TaKaRa (Japan). All unspecified reagents were analytical grade.

1.5 Primer Design

Based on mRNA sequences of SLC7A9 (XM_005692243.1), SLC7A1 (XM_005687542.1), SGLT1 (NM_001009404.1), GLUT2 (XM_004003162.1), and the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) published in GenBank, primers were designed and synthesized by Takara Bio (Dalian) Co., Ltd. Nutrient sensing factor primers T1R1 (XM_005690745.1), T1R2 (Gene ID:102171940), and T1R3 (XM_005690861.1) were designed using Primer 5.0 and synthesized by Sangon Biotech (Shanghai) Co., Ltd. Primer information is shown in Table 2 .

Table 2 Primer information

Primer name	Sequences (5' -3')	Annealing temperature (°C)	Product length (bp)	Genes
SLC7A1-F	ACAGGGGAGGAAGGTGAAGAAC	···		Solute carrier family 7 member 1
SLC7A1-R	AAATAGGCGATTGAAGCAGATGAG	···		SLC7A1
SLC7A9-F	TGGCACCTGCATCATCGT	···		Solute carrier family 7 member 9

Primer name	Sequences (5’ -3’)	Annealing temperature (°C)	Product length (bp)	Genes
SLC7A9-R	TCGCAAGAACGCCACACA	···		SLC7A9
SGLT1-F	CTTTGCCATCATCCTCTTTGTC	···		Sodium-glucose co-trans-porter 1
SGLT1-R	ATCTTGAATGTTCCTCGTCTTCTG	···		SGLT1
GLUT2-F	CCAATGTCTGCTGCTCTTCTTCT	···		Glucose trans-porter 2
GLUT2-R	GCTTCTTCCCTTCTCTTTTCTCATCT	···		GLUT2
T1R1-F	ATGCTGGCTGTTACCTACAAT	···		Taste 1 re-cep-tor mem-ber 1
T1R1-R	CAGGACACGAAAGTTGAGGAG	···		T1R1
T1R2-F	CAGGAGGACTAACAGCCACTAT	···		Taste 1 re-cep-tor mem-ber 2
T1R2-R	GGAAGGAGGAGGAGGGAGAG	···		T1R2
T1R3-F	GGGCTCCGTGAATCCTACACT	···		Taste 1 re-cep-tor mem-ber 3
T1R3-R	ACCTCCCAACATTCCCACTCTT	···		T1R3
GAPDH-F	GGAGCACGAGAAGGAAGAGAGAG	···		Glyceraldehyde-3-phosphate dehy-dro-ge-nase

Primer name	Sequences (5' -3')	Annealing temperature (°C)	Product length (bp)	Genes
GAPDH-R	CCTTGGGGATGGAAATGTGT	···		GAPDH

1.6 Total RNA Extraction and cDNA Synthesis

Total RNA from jejunum and ileum was extracted using the Trizol method. The A260/A280 ratio measured by UV spectrophotometry was between 1.8-2.0, and total RNA quality was evaluated by agarose gel electrophoresis. According to the PrimeScript RT Master Mix kit instructions, total RNA from all samples was reverse-transcribed at the same concentration. The reaction system (total volume 25 L) contained: 5×PrimeScript Buffer 5 L, PrimeScript RT Enzyme Mix 1.25 L, Oligo dT Primer 1.25 L, Random Primer 1.25 L, total RNA 2.5 L, and RNase-free water 13.75 L. Reaction conditions were: 37°C for 15 min, 85°C for 5 s.

1.7 Real-time Quantitative PCR

According to the TaKaRa kit instructions, a two-step reaction was performed: 95°C pre-denaturation for 30 s; 40 cycles of 95°C denaturation for 5 s, 60°C annealing for 30 s. The threshold cycle (Ct) was obtained for each sample. Each gene sample was run in triplicate. Using GAPDH as the internal reference, Ct values for each gene were obtained by real-time PCR. The 2-ΔΔCt method was used to calculate mRNA relative expression levels.

1.8 Data Processing and Analysis

Data were processed using SAS 9.0 software for one-way ANOVA, with Duncan' s multiple comparison test. Results are expressed as mean ± standard deviation. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered highly significant.

Results

2.1 Relative Gene Expression Levels in Jejunum and Duodenum

As shown in Table 3 , under basal dietary conditions (0 g/d G), glucose transporter mRNA relative expression levels exhibited the following patterns: SGLT1 mRNA relative expression levels in jejunum of goats in groups 1 and 4 were significantly lower than in group 7 ($P<0.05$), with no significant difference between these two groups ($P>0.05$). In contrast, basic amino acid transporter mRNA relative expression levels showed more pronounced patterns, with SLC7A1 and SLC7A9 mRNA relative expression levels in groups 1 and 4 being significantly

higher than in group 7 (P<0.05). T1R1 and T1R3 mRNA relative expression levels of nutrient sensing factors in jejunum of groups 1 and 4 were significantly higher than in group 7 (P<0.05), while no significant difference in T1R2 mRNA relative expression level was observed among groups (P>0.05).

When rumen-bypass glucose was increased by 20 g/d (20 g/d G) under basal dietary conditions, SGLT1 and GLUT2 mRNA relative expression levels of glucose transporters in jejunum of groups 2 and 5 were significantly higher than in group 8 (P<0.05). Regarding basic amino acid transporters, SLC7A9 mRNA relative expression level in jejunum of groups 2 and 5 was significantly higher than in group 8 (P<0.05); SLC7A1 mRNA relative expression level in jejunum of group 5 was significantly higher than in groups 2 and 8 (P<0.05), with no significant difference between the latter two groups (P>0.05). Nutrient sensing factor mRNA relative expression levels in jejunum did not show consistent patterns.

When rumen-bypass glucose was increased by 40 g/d (40 g/d G) under basal dietary conditions, SGLT1 mRNA relative expression level of glucose transporter in jejunum of groups 3 and 6 was significantly higher than in group 9 (P<0.05); however, GLUT2 mRNA relative expression level in these groups was significantly lower than in group 9 (P<0.05). Amino acid transporter mRNA relative expression levels in groups 3 and 6 were significantly higher than in group 9 (P<0.05). For nutrient sensing factor mRNA relative expression levels in jejunum, T1R1 and T1R3 mRNA relative expression levels in groups 3 and 6 were significantly higher than in group 9 (P<0.05), while T1R2 mRNA relative expression level showed inconsistent patterns with T1R1 and T1R3 among groups.

Table 3 Effects of dietary nutrient level on mRNA relative expression levels of genes in jejunum of goats

Treatments	SGLT1	GLUT2	SLC7A1	SLC7A9	T1R1	T1R2	T1R3
LN+0 g/d G	0.31±0.02b	1.03±0.16b	1.00±0.26b	3.91±0.21b	1.34±0.16b	1.99±0.07b	1.42±0.10b
LN+NCG+0 g/d G	0.54±0.09b	1.26±0.10b	5.56±0.83a	18.2±0.95a	7.34±0.22a	2.03±0.08b	8.44±0.24a
HN+0 g/d G	1.00±0.05a	1.00±0.08b	1.00±0.10c	1.00±0.06c	1.00±0.03c	1.00±0.05c	1.00±0.07c
LN+20 g/d G	4.51±0.16a	1.86±0.80b	0.94±0.09a	1.35±0.62b	0.73±0.36b	1.92±0.00b	1.32±0.05c
LN+NCG+20 g/d G	2.82±0.03b	1.29±0.61b	1.44±0.03b	2.15±0.34c	0.31±0.05c	1.08±1.01b	1.12±0.03a
HN+20 g/d G	1.00±0.03c	1.00±0.07b	1.00±0.06a	1.00±0.08c	1.00±0.11a	1.00±0.09c	1.00±0.08b
LN+40 g/d G	4.38±0.30a	0.43±0.06b	1.63±0.11a	1.60±0.18b	2.28±0.18a	1.24±0.13a	1.35±0.05a

Treatments	SGLT1	GLUT2	SLC7A1	SLC7A9	T1R1	T1R2	T1R3
LN+NCG+40 g/d G	2.71±0.14a	0.30±0.04b	1.31±0.16b	2.41±0.22a	1.46±0.06b	1.51±0.09c	1.27±0.07a
HN+40 g/d G	1.00±0.02b	1.00±0.09a	1.00±0.06c	1.00±0.05c	1.00±0.04c	1.00±0.07b	1.00±0.08b

In the same column, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while different small letter superscripts mean significant difference (P<0.05), and different capital letter superscripts mean highly significant difference (P<0.01). The same applies to Table 4.

As shown in Table 4, under basal dietary conditions, SGLT1 mRNA relative expression level of glucose transporter in duodenum of groups 1 and 4 was significantly lower than in group 7 (P<0.05), with no significant difference between these two groups (P>0.05). Regarding GLUT2 mRNA relative expression level, group 1 was significantly higher than groups 4 and 7 in duodenum (P<0.05). Basic amino acid transporter and nutrient sensing factor mRNA relative expression levels in duodenum showed consistent patterns, with groups 1 and 4 being higher than group 7.

When rumen-bypass glucose was increased by 20 g/d under basal dietary conditions, SGLT1 mRNA relative expression level of glucose transporter in duodenum of groups 2 and 5 was significantly higher than in group 8 (P<0.05). Conversely, GLUT2 results showed group 8 being higher than groups 2 and 5. Basic amino acid transporter and nutrient sensing factor mRNA relative expression levels in duodenum showed consistent results, with group 5 being significantly higher than groups 2 and 8 (P<0.05).

When rumen-bypass glucose was increased by 40 g/d under basal dietary conditions, GLUT2 mRNA relative expression level of glucose transporter in duodenum of group 3 was significantly lower than in group 9 (P<0.05). No significant differences were observed in amino acid transporter mRNA relative expression levels among groups 3, 6, and 9 (P>0.05). For nutrient sensing factor mRNA relative expression levels, T1R1, T1R2, and T1R3 mRNA relative expression levels in group 6 were significantly higher than in groups 3 and 9 (P<0.05).

Table 4 Effects of dietary nutrient level on mRNA relative expression levels of genes in duodenum of goats

Treatments	SGLT1	GLUT2	SLC7A1	SLC7A9	T1R1	T1R2	T1R3
LN+0 g/d G	0.49±0.37b	1.29±0.14a	3.95±0.08a	2.33±0.19a	1.36±0.36a	1.49±0.10b	1.21±0.12ab
LN+NCG+0 g/d G	0.45±0.90b	0.72±0.12b	1.23±0.97b	1.20±0.05b	18.00±0.83a	3.40±0.15a	1.27±0.22a

Treatments	SGLT1	GLUT2	SLC7A1	SLC7A9	T1R1	T1R2	T1R3
HN+0 g/d G	1.00±0.04a	1.00±0.07a	1.00±0.09c	1.00±0.03b	1.00±0.08d	1.00±0.06d	1.00±0.04b
LN+20 g/d G	1.24±0.09a	0.89±0.09a	0.97±0.06b	1.07±0.05c	6.13±0.79a	4.94±0.06b	5.84±0.15a
LN+NCG+20 g/d G	1.28±0.06a	0.70±0.08b	5.38±0.12a	6.13±0.79a	1.00±0.00d	1.66±0.12c	2.20±0.13a
HN+20 g/d G	1.00±0.05b	1.00±0.03a	1.00±0.06b	1.00±0.08c	2.18±0.09b	1.00±0.07d	1.00±0.04b
LN+40 g/d G	0.67±0.14c	0.82±0.11b	0.99±0.14b	0.75±0.05c	5.79±0.11a	4.03±0.10b	4.87±0.10c
LN+NCG+40 g/d G	0.88±0.08b	0.95±0.04ab	1.22±0.11a	2.41±0.22a	4.66±0.18a	3.29±0.04a	4.19±0.07a
HN+40 g/d G	1.00±0.05b	1.00±0.06a	1.00±0.09b	1.00±0.05c	1.00±0.12d	1.00±0.08d	1.00±0.04b

2.2 Blood Physiochemical Indexes

As shown in Table 5 , under basal dietary conditions, plasma urea nitrogen content in group 4 was significantly lower than in groups 1 and 7 ($P<0.05$), with no significant difference between the latter two groups ($P>0.05$). Serum citrulline content in groups 1 and 4 showed a trend of being lower than in group 7, though the difference was not significant ($P>0.05$). Plasma glucose content in groups 4 and 7 showed a trend of being higher than in group 1, with no significant difference among groups ($P>0.05$). Serum insulin content in groups 4 and 7 was significantly higher than in group 1 ($P<0.05$).

Comparing groups 1 and 4, plasma glucose and serum insulin contents in group 4 were higher than in group 1, indicating that NCG supplementation under low nitrogen feeding conditions could enhance gluconeogenesis. This may be attributed to NCG promoting the synthesis of arginine-family amino acids, thereby upregulating plasma glucose and insulin.

When rumen-bypass glucose was increased by 20 or 40 g/d under basal dietary conditions, plasma glucose and serum insulin contents in groups 2, 3, 5, and 6 were higher than in groups 1 and 4, likely due to exogenous glucose infusion elevating plasma glucose content and consequently increasing serum insulin content.

Table 5 Effects of dietary nutrient level on blood physiochemical indexes of goats

Treatments	PUN (mmol/L)	Serum citrulline (pg/mL)	PG (mmol/L)	INS (mU/L)
LN+0 g/d G	8.11±0.27a	401.20±2.02d	2.39±0.12b	20.61±0.01f
LN+20 g/d G	7.09±0.56d	406.79±3.66cd	2.65±0.24ab	26.34±0.09a
LN+40 g/d G	7.30±0.67cd	410.90±0.80abc	3.23±0.30a	22.94±0.90e
LN+NCG+0 g/d G	7.97±0.56b	401.27±5.12d	2.56±0.18ab	22.90±0.99e
LN+NCG+20 g/d G	7.49±0.25c	406.79±6.56cd	2.82±0.35ab	23.78±0.08c
LN+NCG+40 g/d G	7.14±1.02d	410.90±0.80abc	3.10±0.17a	23.98±0.09c
HN+0 g/d G	8.22±1.03a	407.54±5.84bcd	2.75±0.18ab	23.21±1.29de
HN+20 g/d G	8.12±0.86a	414.25±5.19a	2.85±0.25ab	24.99±5.18b
HN+40 g/d G	8.52±0.31a	415.30±6.22a	3.04±0.24ab	23.31±2.28d

In the same column, values with no letter or the same letter superscripts mean no significant difference (P>0.05), different small letter superscripts mean significant difference (P<0.05), and different capital letter superscripts mean highly significant difference (P<0.01). The same applies to the following table.

2.3 Hormone Contents in Jejunum and Serum

As shown in Table 6 , under basal dietary conditions, jejunal GLP-1 and GLP-2 contents in groups 1 and 4 were significantly lower compared with group 7, indicating that jejunal GLP-1 and GLP-2 contents decreased with increasing dietary nitrogen level (P<0.05). Regarding jejunal GIP and CCK contents, no consistent patterns were observed with changes in dietary nitrogen level.

When rumen-bypass glucose was increased by 20 and 40 g/d, jejunal GLP-1 and GLP-2 contents in groups 2 and 3 were significantly higher than in group 1 (P<0.05), while contents in groups 5 and 6 were also significantly higher than in group 4 (P<0.05). However, results in groups 3 and 6 were lower than in groups 2 and 5, respectively. Similarly, CCK and GIP contents did not show consistent patterns with changes in glucose infusion level.

Table 6 Effects of dietary nutrient level on hormone contents in jejunum and serum of goats

Treatments	Jejunum	Serum					
	GLP-1 (pg/mL)	GLP-2 (ng/mL)	GIP (ng/L)	CCK (ng/L)	GLP-1 (pg/mL)	GLP-2 (ng/mL)	GIP (ng/L)
LN+0 g/d G	55.16±2.14h	5.85±0.12c	41.31±6.25f	70.62±3.76i	73.26±6.23d		
LN+20 g/d G	72.54±3.19e	6.51±0.08a	44.99±7.37e	88.62±6.71h	73.52±7.54d		
LN+40 g/d G	72.09±6.57f	6.37±1.02ab	34.32±6.17g	91.70±5.88g	69.26±0.83de		
LN+NCG+0 g/d G	71.57±9.03g	5.39±0.84e	33.97±3.26g	87.34±6.73a			
LN+NCG+20 g/d G	78.28±8.24d	6.74±0.75ab	47.96±6.52d	81.97±6.04a			
LN+NCG+40 g/d G	74.43±3.03c	6.44±0.24bc	28.55±3.68g	74.44±3.54e			
HN+0 g/d G	80.56±1.73c	6.71±0.83ab	57.75±9.12b	77.71±9.73c			
HN+20 g/d G	97.54±5.92a	5.73±0.04de	60.90±7.36b	87.87±9.53b			
HN+40 g/d G	81.45±5.63b	6.06±0.53cd	56.53±3.46b	83.62±8.74f			

Discussion

3.1 Effects of Dietary Nutrient Level on mRNA Relative Expression Levels of Nutrient Transporters and Intestinal Sensing Factors in Cashmere Goats

The T1R2/T1R3 heterodimer functions as a receptor for sensing intestinal sugars and artificial sweeteners, capable of detecting available glucose in the intestine and promoting secretion of GLP-1 and peptide YY (PYY) in the gastrointestinal tract. Research results indicate that under high glucose conditions, the sweet taste receptor T1R2/T1R3 can promote expression of apical GLUT2 in intestinal epithelium, thereby enhancing glucose absorption capacity. Glucose present in the intestinal lumen can activate T1R2 and T1R3 in enteroendocrine cells (EECs), simultaneously triggering GLP-1 release [22]. EEC hormones influence glucose absorption by upregulating SGLT1 expression and promoting apical GLUT2 expression in intestinal epithelial cells. Consequently, high-carbohydrate diets and sweeteners can upregulate SGLT1 expression dependent on gustducin (Gg) and T1R1 in taste bud tissue [23].

In this experiment, under basal dietary conditions with duodenal infusion of 20 and 40 g/d glucose, nutrient sensing factor T1R2 and T1R3 mRNA relative expression levels in duodenum and jejunum of goats showed an overall increasing trend. Concurrently, glucose transporter SGLT1 mRNA relative expression level also increased correspondingly, consistent with the aforementioned research findings. However, GLUT2 changes did not show consistent patterns, differing from previous results. This discrepancy may be attributed to species differences. For instance, studies have reported that high-glucose diets increase

apical GLUT2 expression in rat intestine, but similar high-glucose diets have not increased apical GLUT2 expression in goats [24].

In animal diets, ingested proteins are hydrolyzed by pepsin and trypsin. Not all proteins must be hydrolyzed into free amino acids for utilization; many protein metabolites can directly enter systemic circulation through gastrointestinal mucosa. This process requires mediation by protein and peptide sensing receptors and transporters. Basic amino acid transporters represent the primary pathway for dietary basic amino acids to move from the intestinal lumen into enterocytes and the circulatory system. Regulating intestinal amino acid transporter expression to provide adequate amino acids is an effective approach to prevent malabsorption. Recent reports indicate that dietary nitrogen levels or protein hydrolysis products such as amino acids regulate amino acid carrier transport through complex metabolic pathways [25-26], primarily by activating protein and peptide sensors that stimulate EECs to release related hormones (CCK, GLP-1), ultimately signaling amino acid transporters to increase their expression and promote amino acid absorption and metabolism in the small intestine.

T1R1/T1R3 is a heterodimeric membrane receptor that senses proteins and their metabolites in mammals. Because T1R1/T1R3 can recognize "umami" flavor produced by glutamate, it is also called the "umami sensor" [12]. Current research indicates this receptor can sense 20 L-amino acids, though sensors in different species show specific responses to amino acid types. For example, rat T1R1/T1R3 responds to all 20 L-amino acids, whereas human T1R1/T1R3 selectively responds only to monosodium glutamate (MSG) and aspartic acid (Asp).

NCG is an analog of N-acetylglutamate (NAG) that participates in the urea cycle in animals. Studies have shown that NCG can promote synthesis of citrulline from glutamine or proline, thereby promoting arginine synthesis [27]. NCG is therefore also called an endogenous activator of arginine. This experiment investigated the effects of functional amino acids on intestinal basic amino acid transporter and nutrient sensing factor expression by adding NCG to low-nitrogen diets, yielding satisfactory results. Under basal dietary conditions, nutrient sensing factor T1R1 and T1R3 mRNA relative expression levels in duodenum and jejunum of goats in the LN+NCG treatment showed an overall trend of being higher than in the LN treatment. It should be noted that this experiment only measured mRNA relative expression levels of individual sensing factors, whereas umami sensors in animals function as heterodimers. Additionally, T1R3 is not only a component of the umami sensor but also a member of the sweet taste receptor (T1R2/T1R3) in the intestine. Therefore, these results only preliminarily indicate that NCG supplementation has some effect on sensing factor expression. More accurate determination would require combination with other methods (such as immunohistochemistry) and other indicators (such as hormones released by small intestinal epithelial endocrine cells), which were not performed in detail in this experiment. Results also showed that basic amino acid transporters SLC7A1 and SLC7A9 mRNA relative expression levels in duo-

denum and jejunum of LN and LN+NCG treatments showed an overall trend of being higher than in the HN treatment. This primarily occurs because reducing dietary nitrogen level in goats can promote endogenous nitrogen synthesis [28], consistent with results showing that decreasing dietary nitrogen level promotes nitrogen metabolism in goats. Between these two treatments, LN+NCG showed higher SLC7A1 and SLC7A9 mRNA relative expression levels in duodenum and jejunum than LN treatment, mainly due to increased expression of sensing factors T1R1 and T1R3. As an "umami sensor," T1R1/T1R3 can be activated by amino acid stimulation, thereby prompting EECs to release CCK and GLP-1, which signal amino acid transporters to increase their expression.

3.2 Effects of Dietary Nutrient Level on Blood Physiochemical Indexes and Hormone Contents in Jejunum and Serum of Cashmere Goats

This experiment measured plasma glucose and serum insulin contents to roughly infer the effects of feeding different nitrogen levels, different duodenal glucose infusion levels, and NCG supplementation on glucose metabolism in goats. Plasma glucose in ruminants is primarily regulated by hepatic gluconeogenesis, with dietary regulation being secondary. Results showed that when glucose was infused into the duodenum, plasma glucose and serum insulin contents in LN and LN+NCG treatments tended to be lower than in the HN treatment, possibly due to weakened hepatic gluconeogenesis [29]. In low-nitrogen feeding systems, weakened gluconeogenesis is mainly based on reduced non-essential amino acids such as citrulline [30]. In this experiment, under basal dietary conditions, serum citrulline contents in LN and LN+NCG treatments showed a trend of being lower than in the HN treatment. This result further supports the above inference. It is well known that citrulline is involved not only in urea synthesis but also in gluconeogenesis. Significantly reducing dietary nitrogen level in rats markedly decreased hepatic citrulline synthesis, with corresponding decreases in blood glucose and insulin contents [31-32]. This conclusion aligns with Lariviere et al.' s [33] findings in humans consuming severely protein-deficient diets. In this experiment, three different glucose levels (0, 20, and 40 g/d) were infused into goat duodenum. When 20 and 40 g/d glucose were infused, plasma glucose and serum insulin contents in groups 2 and 5 showed an overall trend of being higher than in group 8. It can be inferred that as dietary nitrogen level decreases, plasma amino acid content also decreases, negatively affecting gluconeogenesis in cashmere goats and causing plasma glucose content to decline, which consequently reduces secretion of glucose-dependent hormones. Adding NCG to low-nitrogen diets effectively increased plasma amino acid and related hormone contents, further confirming that NCG can promote synthesis of non-essential amino acids in the body.

Research has confirmed that sweeteners (glucose, sucrose, and sucralose) promote GLP release from intestinal endocrine cell line NCI-716 in a dose-dependent manner. Meanwhile, GLP-1 release in the intestine depends on

SGLT1 participation [34]. In rats with T1R3 gene knockout, sweetener supplementation did not induce GLP-1 and GIP secretion [23,35]. This experiment showed that serum GLP-1 contents in HN, LN, and LN+NCG treatments increased after 20 and 40 g/d glucose infusion compared with basal dietary levels, with LN+NCG+20 g/d G treatment showing the highest value. These results are consistent with the aforementioned studies. On one hand, increased glucose content in the small intestine may elevate plasma glucose content, thereby increasing glucose-dependent hormones (GLP-1 and GLP-2). On the other hand, this may be due to the highest SGLT1 and T1R3 mRNA relative expression levels in goats in this treatment. In summary, duodenal glucose infusion under low-nitrogen dietary conditions can alleviate the decrease in plasma glucose content. However, excessive glucose infusion did not significantly increase glucose-dependent hormone content, possibly because the body cannot completely absorb excessive rumen-bypass glucose. Appropriate glucose infusion can improve the metabolic effects of low nitrogen, and the impact of NCG supplementation on plasma glucose, GLP-1, and GLP-2 becomes more pronounced with increased rumen-bypass glucose.

Protein metabolites are potent stimulators of CCK release, with protein having a much stronger CCK-stimulating effect than carbohydrates [36]. After protein metabolites (such as peptides and amino acids) stimulate gastrointestinal "umami sensors," intracellular calcium concentration in taste cells increases, leading to CCK, PYY, and GLP release. These gut hormones can activate the vagus nerve and corresponding target cells [37]. However, results in this study did not align with this conclusion, as CCK content did not show consistent patterns with changes in dietary nitrogen level, glucose infusion level, or NCG supplementation. This may be because CCK is primarily secreted in the duodenum, whereas this experiment measured its content in jejunal tissue. Research on this aspect remains inconclusive.

In conclusion, appropriately reducing dietary nitrogen level, supplementing NCG, and increasing rumen-bypass glucose (duodenal infusion of 20 g/d) can promote metabolism and intestinal nutrient sensing in cashmere goats.

References

[1] RAYBOULD H E. Gut chemosensing: interactions between gut endocrine cells and visceral afferents[J]. Autonomic Neuroscience, 2010, 153(1/2): 41-46.

[2] MCINTYRE N, HOLDSWORTH C D, LEEDS M B, et al. New interpretation of oral glucose tolerance[J]. The Lancet, 1964, 284(7349): 20-21.

[3] LAM C K L, CHARI M, LAM T K T. CNS regulation of glucose homeostasis[J]. Physiology, 2009, 24(3): 159-170.

[4] LIU Y, IPHARRAGUERRE I R, PETTIGREW J E. Digestive physiology of the pig symposium: potential applications of knowledge of gut chemosensing

in pig production[J]. Journal of Animal Science, 2013, 91(5): 1982-1990.

[5] HUNTINGTON G B. Starch utilization by ruminants: from basics to the bunk[J]. Journal of Animal Science, 1997, 75(3): 852-867.

[6] HARMON D L, MCLEOD K R. Glucose uptake and regulation by intestinal tissues: implications for whole-body energetics[J]. Journal of Animal Science, 2001, 79(E-Suppl): E59-E72.

[7] 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(1): 35-54.

[8] WU S V, ROZENGURT N, YANG M, et al. Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells[J]. Proceedings of the National Academy of Sciences of the United States of America, 2002, 99(4): 2392-2397.

[9] ROZENGURT E, STERNINI C. Taste receptor signaling in the mammalian gut[J]. Current Opinion in Pharmacology, 2007, 7(6): 557-562.

[10] YOUNG R L. Sensing via intestinal sweet taste pathways[J]. Frontiers in Neuroscience, 2011, 5: 23.

[11] BURANT C F, TAKEDA J, BROT-LAROCHE E, et al. Fructose transporter in human spermatozoa and small intestine is GLUT5[J]. The Journal of Biological Chemistry, 1992, 267(21): 14523-14526.

[12] ZHAO G Q, ZHANG Y F, HOON M A, et al. The receptors for mammalian sweet and umami taste[J]. Cell, 2003, 115(3): 255-266.

[13] ZHAO Hongli. Effects of arginine on intestinal protein synthesis, amino acid transporter mRNA expression and intestinal health in fine-wool sheep[D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2012.

[14] LI Jinxia. Effects of arginine and soybean oil on skeletal muscle protein synthesis and intramuscular fat content changes in fine-wool sheep[D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2011.

[15] SUN Haizhou. Study on the overall optimization law of glucose nutrition in growing and fattening sheep[D]. PhD thesis. Hohhot: Inner Mongolia Agricultural University, 1999.

[16] HAN Fei. Study on the determination of propionic acid production and absorption rate of common ruminant feeds and their models[D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2001.

[17] REN Ying. Study on the determination of rumen-bypass starch content and small intestinal digestibility of common ruminant feeds and related technologies[D]. Master' s thesis. Nanning: Guangxi University, 2001.

[18] WANG Ling. Evaluation of appropriate metabolic glucose level in Inner Mongolia cashmere goats[D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2003.

[19] NRC. Nutrient requirements of dairy cattle[S]. 7th ed. Washington, D.C.: National Academy of Sciences, 2001.

[20] ALDERMAN J. Energy and protein requirements of ruminants: an advisory manual prepared by the AFRC technical committee on responses to nutrients[M]. Wallingford: CABI Publishing, 1993.

[21] LI Mei. American Feedstuffs feed ingredient analysis table (2007 edition)[J]. Feed China, 2007(12): 37-40.

[22] KOKRASHVILI Z, RODRIGUEZ D, YEVSHAYEVA V, et al. Release of endogenous opioids from duodenal enteroendocrine cells requires Trpm5[J]. Gastroenterology, 2009, 137(2): 598-606.e2.

[23] MARGOLSKEE R F, DYER J, KOKRASHVILI Z, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1[J]. Proceedings of the National Academy of Sciences of the United States of America, 2007, 104(38): 15075-15080.

[24] KELLETT G L. The facilitated component of intestinal glucose absorption[J]. The Journal of Physiology, 2001, 531(3): 585-595.

[25] KARASOV W H, SOLBERG D H, CHANG S D, et al. Is intestinal transport of sugars and amino acids subject to critical-period programming?[J]. American Journal of Physiology-Gastrointestinal and Liver Physiology, 1985, 249(6): G770-G785.

[26] KARASOV W H, SOLBERG D H, DIAMOND J M. Dependence of intestinal amino acid uptake on dietary protein or amino acid levels[J]. American Journal of Physiology: Gastrointestinal and Liver Physiology, 1987, 252(5): G614-G625.

[27] WU G Y, KNABE D A, KIM S W. Arginine nutrition in neonatal pigs[J]. The Journal of Nutrition, 2004, 134(10S): 2783S-2390S.

[28] COLMENERO J J O, BRODERICK G A. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows[J]. Journal of Dairy Science, 2006, 89(5): 1704-1712.

[29] MUSCHER-BANSE A S, PIECHOTTA M, SCHRÖDER B, et al. Modulation of intestinal glucose transport in response to reduced nitrogen supply in young goats[J]. Journal of Animal Science, 2013, 90(13): 4995-5004.

[30] MARKANTONATOS X, GREEN M H, VARGA G A. Use of compartmental analysis to study ruminal volatile fatty acid metabolism under steady state conditions in Holstein heifers[J]. Animal Feed Science and Technology, 2008, 143(1/2/3/4): 70-88.

[31] KORITZ S B, COHEN P P. The effect of diet on citrulline synthesis in vitro[J]. The Journal of Biological Chemistry, 1953, 200(2): 551-557.

[32] MCGIVAN J D, BRADFORD N M, MENDES-MOURÃO J. The regulation of carbamoyl phosphate synthase activity in rat liver mitochondria[J]. Biochemical Journal, 1976, 154(2): 415-421.

[33] LARIVIÈRE F, CHIASSON J L, SCHIFFRIN A, et al. Effects of dietary protein restriction on glucose and insulin metabolism in normal and diabetic humans[J]. Metabolism, 1994, 43(4): 462-467.

[34] MACE O J, AFFLECK J, PATEL N, et al. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2[J]. The Journal of Physiology, 2007, 582(1): 379-392.

[35] JANG H J, KOKRASHVILI Z, THEODORAKIS M J, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1[J]. Proceedings of the National Academy of Sciences of the United States of America, 2007, 104(38): 15069-15074.

[36] TAN Bi' e, YIN Yulong. Gastrointestinal nutrient chemosensing and its physiological effects[J]. Chinese Journal of Animal Nutrition, 2013, 25(2): 231-241.

[37] SUTHERLAND K, YOUNG R L, COOPER N J, et al. Phenotypic characterization of taste cells of the mouse small intestine[J]. American Journal of Physiology: Gastrointestinal and Liver Physiology, 2007, 292(5): G1420-G1428.

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