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Original Research Article

Low rumen degradable starch promotes the growth performance of goats by increasing protein synthesis in skeletal muscle via the AMPK-mTOR pathway

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ABSTRACT

Since starch digestion in the small intestine provides more energy than digestion in the rumen of ruminants, reducing dietary rumen degradable starch (RDS) content is beneficial for improving energy utilization of starch in ruminants. The present study tested whether the reduction of rumen degradable starch by restricting dietary corn processing for growing goats could improve growth performance, and further investigated the possible underlying mechanism. In this study, twenty-four 12-wk-old goats were selected and randomly allocated to receive either a high RDS diet (HRDS, crushed corn-based concentrate, the mean of particle sizes of corn grain = 1.64 mm, $n = 12$) or a low RDS diet (LRDS, non-processed corn-based concentrate, the mean of particle sizes of corn grain >8 mm, $n = 12$). Growth performance, carcass traits, plasma biochemical indices, gene expression of glucose and amino acid transporters, and protein expression of the AMPK-mTOR pathway were measured. Compared to the HRDS, LRDS tended to increase the average daily gain (ADG, $P = 0.054$) and decreased the feed-to-gain ratio (F/G, $P < 0.05$). Furthermore, LRDS increased the net lean tissue rate ($P < 0.01$), protein content ($P < 0.05$) and total free amino acids ($P < 0.05$) in the biceps femoris (BF) muscle of goats. LRDS increased the glucose concentration ($P < 0.01$), but reduced total amino acid concentration ($P < 0.05$) and tended to reduce blood urea nitrogen (BUN) concentration ($P = 0.062$) in plasma of goats. The mRNA expression of insulin receptors (*INSR*), glucose transporter 4 (*GLUT4*), L-type amino acid transporter 1 (*LAT1*) and 4F2 heavy chain (*4F2hc*) in BF muscle, and sodium-glucose cotransporters 1 (*SGLT1*) and glucose transporter 2 (*GLUT2*) in the small intestine were significantly increased ($P < 0.05$) in LRDS goats. LRDS also led to marked activation of p70-S6 kinase (S6K) ($P < 0.05$), but lower activation of AMP-activated protein kinase (AMPK) ($P < 0.05$) and eukaryotic initiation factor 2α ($P < 0.01$). Our findings suggested that reducing the content of dietary RDS enhanced postruminal starch digestion and increased plasma glucose, thereby improving amino acid utilization and promoting protein synthesis in the skeletal muscle of goats via the AMPK-mTOR pathway. These changes may contribute to improvement in growth performance and carcass traits in LRDS goats.

1. Introduction

With the expansion of global production and consumption of milk and meat products, starch-enriched diets are usually formulated during production in order to meet the energy demands of ruminants for fast growth and high yield (Ren et al., 2020). However, high-starch diets, especially high rumen degradable starch (RDS) diets, may also increase the risk of rumen acidosis and affect animal health (Nagaraja and Titgemeyer, 2007; Stefanska et al.,

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2018). In addition, compared with starch digested in the small intestine, starch digested in the rumen of ruminants produces volatile fatty acids (VFA) and methane, resulting in energy loss (Huntington et al., 2006; Harmon, 2009; Owens et al., 2016). Therefore, reducing the RDS in the diet is beneficial to rumen health and improves the energy utilization of starch in ruminants (Theurer, 1986).

Dietary starch in ruminants is mainly divided into RDS and rumen escape starch according to their degradation sites. The RDS content can be regulated by adjusting the grain formula, changing the grain processing method and feeding method (Theurer, 1986; Li et al., 2014; Zheng et al., 2020). Corn, a rich source of starch, is one of the most commonly used energy feeds for ruminants (Richards and Hicks, 2007). Increasing corn particle size reduces dietary RDS content (Callison et al., 2001; Rémond et al., 2004) and studies have shown that increasing the extent of grain processing in the diet was not conducive to improving the growth performance of ruminants (Owens et al., 1997; Gorocica-Buenfil and Loerch, 2005; de Melo et al., 2019). Therefore, strategies to reduce dietary RDS by limiting grain processing in diets are critically important for improving the growth and production performance of ruminants.

The AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways are interrelated and coordinate cell growth and metabolism with energy and nutrient inputs (Lin and Hardie, 2018; Gonzalez et al., 2020). AMPK is activated when the energy supply is insufficient and maintains a balance between energy supply and demand by promoting the catabolic pathway, leading to ATP generation and inhibiting the anabolic pathway that consumes ATP (Hardie et al., 2016; Carling, 2017; Gonzalez et al., 2020). In contrast, once activated when nutrients are available, mTOR acts to inhibit the catabolic pathway and promote anabolic pathways, such as protein synthesis, and lipid, nucleotide and glucose metabolism, resulting in cell growth (Saxton and Sabatini, 2017). A study has shown that whole corn diets (low RDS diets [LRDS]) have higher metabolizable energy content than dry-rolled corn diets (Owens et al., 1997). Therefore, we assumed that the AMPK-mTOR pathway is involved in the growth of ruminants promoted by LRDS diets.

Here, we hypothesized that reducing dietary RDS content would increase the postruminal starch digestion and improve the energy utilization of starch, thereby promoting protein synthesis of goats through the AMPK-mTOR pathway and result in better growth performance. Our study aimed to test whether reducing dietary RDS content of growing goats could improve growth performance and further assess the role of the AMPK-mTOR pathway in this process to reveal the possible underlying mechanism.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Institutional Animal Care and Use Committee of Northwest A&F University (NWAFAC1008).

2.2. Experimental design and diets

Twenty four male 3-mo Saanen goats (13.6 ± 0.23 kg BW) were randomly selected to receive either a high rumen degradable starch diet (HRDS, crushed corn-based concentrate, the mean of particle sizes of corn grain = 1.64 mm, $n = 12$) or LRDS (non-processed corn-based concentrate, the mean of particle sizes of corn grain >8 mm, $n = 12$). The nutritional components of the 2 diets (Table 1) were the same. The corn particle size distribution is shown in Table S1. Goats under ad libitum access to feed and water were fed twice daily at 08:00 and 16:00. The pre-feeding period was 1 wk, and the trial period was 28 wk.

Table 1
Ingredients of the experimental diet (dry matter basis, %).

Item	HRDS	LRDS
Ingredients		
Whole corn		20.9
Crushed corn	20.9	
Corn silage	20.6	20.6
Alfalfa hay	39.9	39.9
Wheat bran	10.7	10.7
Soybean meal	5.9	5.9
Premix[1]	0.1	0.1
Salt	1.3	1.3
$CaHPO_4$	0.3	0.3
$NaHCO_3$	0.3	0.3
Nutrient levels[2]		
DM	57.4	57.4
Crude protein	15.8	15.6
Starch	23.5	24.7
NDF	30.4	30.5
ADF	16.0	15.6

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] The premix contains (per kilogram): 2,925 mg of Cu (as $CuSO_4 \cdot 5H_2O$), 3,900 mg of Fe as $FeSO_4 \cdot H_2O$), 2,750 mg of Zn (as $ZnSO_4 \cdot H_2O$), 800 mg of Mn (as $MnSO_4 \cdot H_2O$).
[2] All nutrient levels were the measured value.

2.3. Sample collection

Analyses performed on feed samples were as follows: dry matter, according to official method 934.01 (AOAC, 1995); crude protein (CP), according to official method 976.05 (AOAC, 1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and starch (Megazyme, Bray, Ireland) were according to Van Soest et al. (1991). Daily feed intake was recorded on an individual goat basis for 3 consecutive days once a week throughout the experimental period to measure average daily feed intake (ADFI). Average daily gain (ADG) was measured by recording body weight on the day at the start and end of the experiment. The feed-to-gain ratio (F/G) was calculated. At wk 25 to 26, 6 goats from each group were picked randomly and placed in metabolic cages for a digestibility trial. Urine from each goat was collected in a bucket every day before morning feeding, mixed with 100 mL of 3.6 mol/L H_2SO_4, and stored at −20 °C for microbial protein (MCP) synthesis (Wojciechowski and Barbano, 2015) and purine derivative analyses (Westreicher-Kristen et al., 2020). Before the morning fed in the last 3 days of wk 27, jugular venous blood was collected, and then immediately centrifuged (3,000 × g at 4 °C for 15 min) to obtain the plasma required for further analysis. At the end of wk 28, 8 goats from each group were randomly selected, weighed, and slaughtered in pairs by bloodletting from the neck. All skeletal muscles were separated and weighed to calculate the lean tissue weight. Samples of liver, jejunum and biceps femoris (BF) muscle were taken and placed in sterile cryogenic vials, frozen in liquid nitrogen and stored at −80 °C for subsequent analysis. Crude protein of skeletal muscle was determined by the Kjeldahl method according to Needham et al. (2019).

2.4. Measurement of the concentration of free amino acids (AA)

An Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA) was used to measure the plasma amino acid concentrations of goats. Quantification was performed by an external standard method. Four hundred microliter of 10% sulfosalicylic acid was added to the plasma samples (100 μL) to remove protein and the supernatant was obtained for the assay of amino acids. Fifty milligram freeze-dried BF muscle tissue was homogenized by ultrasound with 1 mL 0.01 mol/L HCl (pH = 2) and then centrifuged (10,000 × g at 4 °C for 10 min) to obtain the supernatant. The

subsequent measurement method was the same as that of plasma AA.

2.5. RNA extraction and quantitative real-time PCR analysis

Total RNA was extracted from each tissue sample using TRIzol reagent (9109; Taraka, Osaka, Japan). The concentration and quality of the RNA were assessed with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). A 10 μL reaction volume containing 1 μg of RNA was used for cDNA synthesis, using the PrimeScript first-strand cDNA synthesis kit (6210A Takara, Osaka, Japan). Real-time PCR was performed in a Roche LightCycler 96 qPCR system (Roche, Basel, Switzerland) using the TB Green qPCR kit (RR820A, Taraka, Osaka, Japan). The primers used for quantitative real-time PCR are shown in Table S2. Beta-actin and GAPDH were chosen as the reference genes. The gene expression was measured by quantitative real-time PCR analysis as described previously (Wang et al., 2020).

2.6. Western blot analysis

Approximately 50 mg of frozen BF muscle was taken and homogenized in 200 μL of ice-cold RIPA lysis buffer (Solarbio, Beijing, China) containing 1 mmol/L PMSF, 5 mmol/L NaF and 1 mmol/L Na_3VO_4. The homogenates were shaken on ice for 30 min and then centrifuged (12,000 × *g* at 4 °C for 15 min) to obtain the supernatant. Ten microliters of supernatant was taken to determine protein concentration using a BCA Protein Assay kit (Tiangen, Beijing, China). The remaining supernatant was diluted to the proper protein concentration with RIPA buffer and then mixed with 5 × loading buffer (CWBIO, Beijing, China) and finally boiled at 100 °C for 10 min to obtain the samples.

The protein expression was measured by Western blot analysis as described previously (Guo et al., 2018). Beta-tubulin was chosen as the reference protein, and the anti-β-tubulin antibody (1:2,000; catalog number 10094-1-AP) was purchased from Proteintech Group (Rosemont, IL 60018, USA). The antibodies including anti-AMPKα, anti-p-AMPKα, anti-p70S6K, anti-p-p70S6K, anti-eIF2α and anti-p-eIF2a (1:100; catalog numbers 2532, 2531, 9202, 9205, 9722, and 9721, respectively) were purchased from Cell Signaling Technology (Danvers, MA, USA). The secondary antibodies used were goat anti-rabbit or anti-mouse HRP-conjugated (1:4,000; Bioworld Technology).

2.7. Statistical analysis

Outlying results were identified by Grubbs' test and were tested for normal distribution, followed by the Student's unpaired t-test using the SPSS software package (SPSS version 25; SPSS Inc., Chicago, IL, USA). The growth performance of one goat in each group was identified as an outlier using Grubbs test, and both goats were excluded. The data are presented as the mean ± standard error of the mean (SEM). Significance was defined as $P < 0.05$, and trends were defined as $0.05 \leq P < 0.10$.

3. Results

3.1. Growth performance and carcass traits of goats

The growth performance and carcass traits are shown in Table 2. In comparison to HRDS, LRDS had no significant effect on ADFI ($P > 0.10$), but tended to increase the ADG ($P = 0.054$) and significantly decreased the F/G ($P < 0.05$). As expected, LRDS significantly increased the net meat rate ($P < 0.01$, Table 3) and carcass net meat rate of goats ($P < 0.01$, Table 3), compared to the HRDS group. However, no difference was observed in the dressing percentage ($P > 0.10$, Table 3) among these 2 groups.

Table 2
Growth performance of goats ($n = 11$).

Item	HRDS	LRDS	SEM	*P*-value
ADFI, g/d	706.57	736.12	25.14	0.572
ADG, g/d	75.71	92.51	4.40	0.054
F/G, g/g	9.78	8.08	0.41	0.037

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; ADFI = average daily feed intake; ADG = average daily gain; F/G = the feed-to-gain ratio.

Table 3
Carcass traits of goats ($n = 7$).

Item	HRDS	LRDS	SEM	*P*-value
Live weight, kg	33.21	33.74	2.09	0.805
Carcass weight, kg	15.49	15.83	1.29	0.795
Dressing percentage, %	46.35	46.88	1.20	0.663
Lean tissue weight, kg	10.73	12.33	0.92	0.107
Lean tissue weight to live weight, %	32.21	36.52	1.35	0.008
Lean tissue weight to carcass weight, %	69.56	77.91	2.56	0.007

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch.

Table 4
Plasma biochemical indices of goats ($n = 7$).

Item	HRDS	LRDS	SEM	*P*-value
GLU, mmol/L	1.68	1.96	0.06	0.007
BUN, mmol/L	6.74	5.33	0.38	0.062
Insulin, μIU/mL	4.89	5.63	0.52	0.500

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; GLU = glucose; BUN = blood urea nitrogen.

3.2. Plasma biochemical indices in goats

The plasma glucose level was significantly increased ($P < 0.01$, Table 4) in the LRDS goats, compared to the HRDS group. By contrast, a trend for decreased blood urea nitrogen (BUN) was observed in LRDS goats ($P = 0.062$, Table 4) compared to the HRDS group. There was little difference observed ($P > 0.10$, Table 4) in insulin concentrations in plasma.

3.3. Gene expression of gluconeogenesis enzymes in the liver of goats

As shown in Fig. 1, no significant difference ($P > 0.10$) was observed in the mRNA levels of gluconeogenesis enzymes glucose-6-phosphatase (G6P), phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase (PC) among the 2 dietary treatments.

3.4. MCP synthesis and crude protein in skeletal muscle of goats

As shown in Table 5, there was no significant difference in purine derivatives (including xanthine, hypoxanthine, trioxypurine and allantoin, $P > 0.10$) and MCP ($P > 0.10$) among these 2 groups. Table 6 shows that the absolute-dry weight ($P < 0.01$) and crude protein ($P < 0.05$) in skeletal muscle were significantly increased in LRDS goats, compared to the HRDS group. No significant difference ($P > 0.10$, Table 6) was observed in protein concentrations in skeletal muscle between these 2 groups.

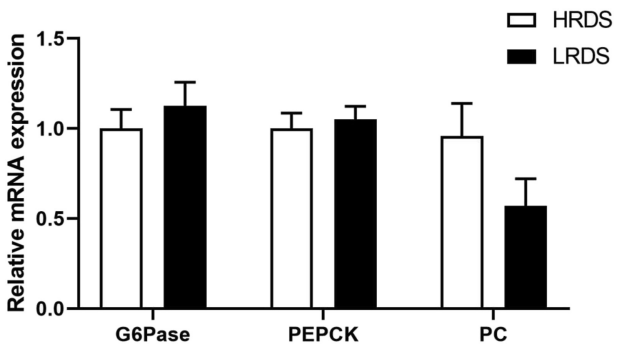

Fig. 1. Effects of rumen degradable starch (RDS) on gene expression of gluconeogenesis enzyme in the liver of growing goats. The mRNA expression levels of *G6Pase*, *PEPCK* and *PC* were normalized to β-actin and *GAPDH* mRNA. HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; G6Pase = glucose-6-phosphatase; PEPCK = phosphoenolpyruvate carboxykinase; PC = pyruvate carboxylase. Results are shown as mean ± SEM, $n = 7$.

Table 5
Purine derivative excretion in urine and MCP synthesis in the rumen of goats ($n = 6$).

Item	HRDS	LRDS	SEM	*P*-value
Xanthine + hypoxanthine, mmol/d	0.48	1.22	0.27	0.173
Trioxypurine, mmol/d	1.16	1.14	0.21	0.963
Allantoin, mmol/d	6.03	7.07	0.84	0.559
PD, mmol/d	7.67	9.44	1.09	0.440
MBN, g/d	6.14	7.59	0.94	0.465
MCP, g/d	38.41	47.42	5.87	0.465

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; PD = purine derivatives; MBN = microbial biomass nitrogen; MCP = microbial protein.

Table 6
Crude protein in skeletal muscle of goats ($n = 7$) (DM basis).

Item	HRDS	LRDS	SEM	*P*-value
Skeletal muscle weight, g	2,979	4,342	211	<0.001
Protein concentrations, g/100 g	60.82	62.87	1.24	0.433
Crude protein in skeletal muscle, g	1,805	2,716	184	0.040

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch.

3.5. Free amino acid concentrations in the plasma and BF muscle of goats

LRDS significantly decreased the individual non-essential AA (NEAA) concentrations including Cys, Pro and Gly ($P < 0.05$, Table 7) and resulted in lower concentrations of total NEAA ($P < 0.05$, Table 7) in the plasma of goats, compared to the HRDS group. LRDS also significantly decreased the individual essential AA (EAA) concentrations including Arg, His, Lys and Met, resulting in lower total AA concentration ($P < 0.01$, Table 7) in the plasma of goats. As shown in Table 8, LRDS significantly increased the concentrations of free EAA (particularly Arg, $P < 0.05$) and total free AA ($P < 0.05$), and tended to increase the concentrations of free branched-chain AA (BCAA) ($P < 0.10$) in BF muscle in goats, compared to the HRDS group.

3.6. Gene expression of glucose and amino acid transporters in the jejunum and BF muscle of goats

The mRNA levels of glucose transporter genes in the jejunum and BF muscle of the goats are shown in Fig. 2. LRDS goats showed higher glucose transporter mRNA levels of both sodium-glucose cotransporters 1 (*SGLT1*) and glucose transporter 2 (*GLUT2*) in the jejunum than the HRDS group ($P < 0.05$, Fig. 2A). As shown in Fig. 2B, insulin receptors (*INSR*) ($P < 0.05$) and glucose transporter 4

Table 7
Plasma free amino acid concentrations in goats ($n = 7$, μmol/L).

Amino acids[1]	HRDS	LRDS	SEM	*P*-value
Arg	387.80	271.40	22.38	0.004
His	501.36	406.16	18.88	0.005
Ile	229.95	266.29	16.75	0.296
Leu	346.44	398.64	21.51	0.239
Lys	167.17	91.93	13.87	0.002
Met	501.36	406.16	18.88	0.005
Phe	72.87	88.50	5.75	0.184
Thr	175.14	157.45	8.38	0.310
Val	573.96	559.36	29.27	0.814
Asp	271.57	333.14	19.35	0.114
Ala	428.12	363.82	25.19	0.214
Glu	91.22	89.60	3.43	0.824
Ser	158.18	94.80	18.69	0.090
Tyr	131.34	119.90	6.17	0.375
Cys	193.32	122.54	17.37	0.035
Trp	126.09	126.93	7.78	0.959
Pro	240.81	155.88	18.31	0.013
Gly	1,133.24	726.80	97.35	0.030
Total EAA	2,956.04	2,645.88	114.29	0.185
Total NEAA	2,773.88	2,133.42	155.10	0.032
Total BCAA	1,150.36	1,224.29	62.51	0.575
Total AA	5,729.90	4,779.30	247.27	0.050

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; total AA = total amino acid; total EAA = total essential amino acid; NEAA = non-essential amino acid; BCAA = total branched-chain amino acid.
[1] Total EAA includes Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val; total NEAA includes Asp, Ala, Glu, Ser, Tyr, Cys, Trp, Pro, and Gly; total BCAA includes Ile, Leu, and Val.

Table 8
Free amino acid concentrations of biceps femoris (BF) muscle in goats ($n = 5$, mg/100 g).

Amino acids[1]	HRDS	LRDS	SEM	*P*-value
Arg	4.57	10.08	1.26	0.016
His	5.27	5.39	0.53	0.918
Ile	0.12	0.16	0.01	0.112
Leu	0.21	0.24	0.02	0.271
Lys	0.13	0.14	0.00	0.566
Met	0.13	0.20	0.04	0.356
Phe	0.21	0.23	0.02	0.646
Thr	0.13	0.16	0.01	0.145
Val	0.20	0.27	0.02	0.082
Asp	2.82	2.98	0.20	0.709
Ala	6.25	7.97	0.59	0.151
Glu	0.44	0.62	0.06	0.139
Ser	0.66	0.75	0.07	0.555
Tyr	0.13	0.17	0.01	0.086
Cys	0.50	0.53	0.06	0.825
Trp	0.25	0.29	0.02	0.414
Pro	0.18	0.26	0.03	0.146
Gly	1.06	0.88	0.12	0.499
Total EAA	10.98	16.88	1.37	0.019
Total NEAA	12.28	14.45	0.84	0.216
Total BCAA	0.53	0.68	0.04	0.089
Total AA	23.26	31.33	1.92	0.024

HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; total AA = total amino acid; total EAA = total essential amino acid; NEAA = non-essential amino acid; BCAA = total branched-chain amino acid.
[1] Total EAA includes Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val; total NEAA includes Asp, Ala, Glu, Ser, Tyr, Cys, Trp, Pro, and Gly; total BCAA includes Ile, Leu, and Val. The free amino acid concentrations were measured and calculated on the freeze-dried weight basis. The 'mg/100 g' means the amino acid content per 100 g of freeze-dried BF muscle.

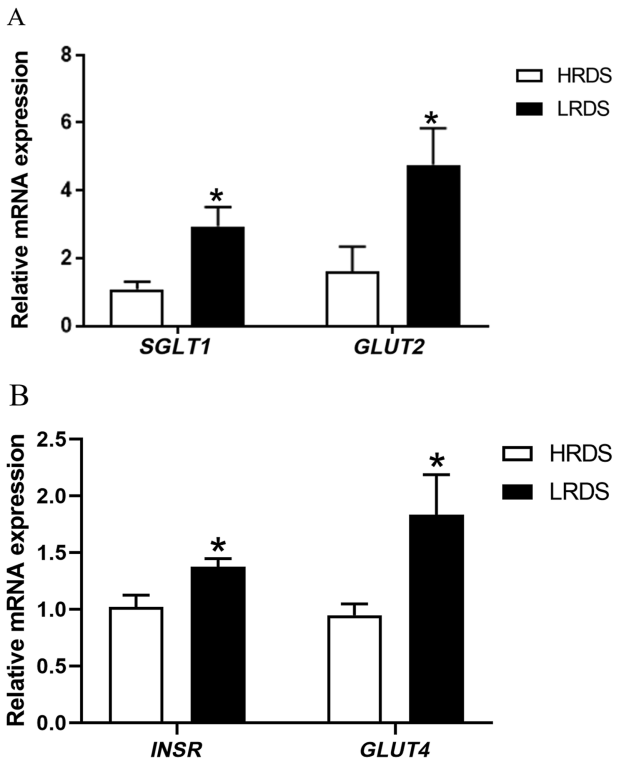

Fig. 2. Effects of rumen degradable starch (RDS) on gene expression of glucose and transporter genes in the jejunum (A, $n = 7$) and biceps femoris (BF) muscle (B, $n = 5$) of growing goats. The mRNA expression levels of *SGLT1*, *GLUT2*, *INSR* and *GLUT4* were normalized to β-actin and *GAPDH* mRNA. HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; *SGLT1* = sodium-glucose cotransporters 1; *GLUT2* = glucose transporter 2; *INSR* = insulin receptors; *GLUT4* = glucose transporter 4. Results are shown as mean ± SEM; *, $P < 0.05$, compared with the HRDS.

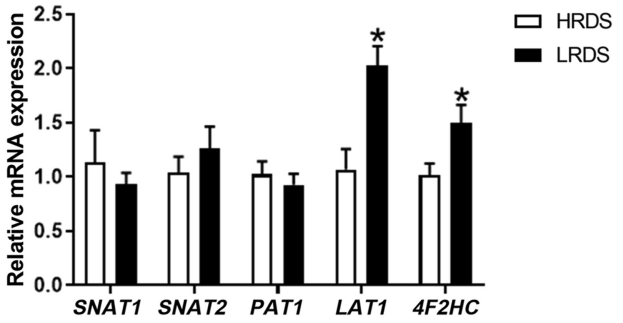

Fig. 3. Effects of rumen degradable starch (RDS) on gene expression of amino acid transporters in the biceps femoris (BF) muscle of growing goats. Level of *SNAT1*, *SNAT2*, *PAT1*, *PAT2*, *LAT1* and *4F2hc* mRNA abundance normalized to β-actin and *GAPDH* mRNA. HRDS = high rumen degradable starch; LRDS = low rumen degradable starch; *SNAT1* = sodium-coupled neutral amino acid transporter 1; *SNAT2* = sodium-coupled neutral amino acid transporter 2; *PAT1* = proton-coupled amino acid transporter 1; *PAT2* = proton-coupled amino acid transporter 2; *LAT1* = L-type amino acid transporter 1; *4F2hc* = 4F2 heavy chain. Results are shown as mean ± SEM, $n = 5$; *, $P < 0.05$, compared with the HRDS.

(*GLUT4*) ($P < 0.05$) mRNA abundance were increased in the BF muscle of LRDS goats compared to the HRDS group. Fig. 3 showed that LRDS significantly increased the gene expression of amino acid transporters L-type amino acid transporter 1 (*LAT1*) ($P < 0.05$) and 4F2 heavy chain (*4F2hc*) ($P < 0.05$), but had no significant effect ($P > 0.10$) on the gene expression of sodium-coupled neutral amino acid transporter 1 (*SNAT1*), sodium-coupled neutral amino acid transporter 2 (*SNAT2*) and proton-coupled amino acid transporter (*PAT*), compared to the HRDS group.

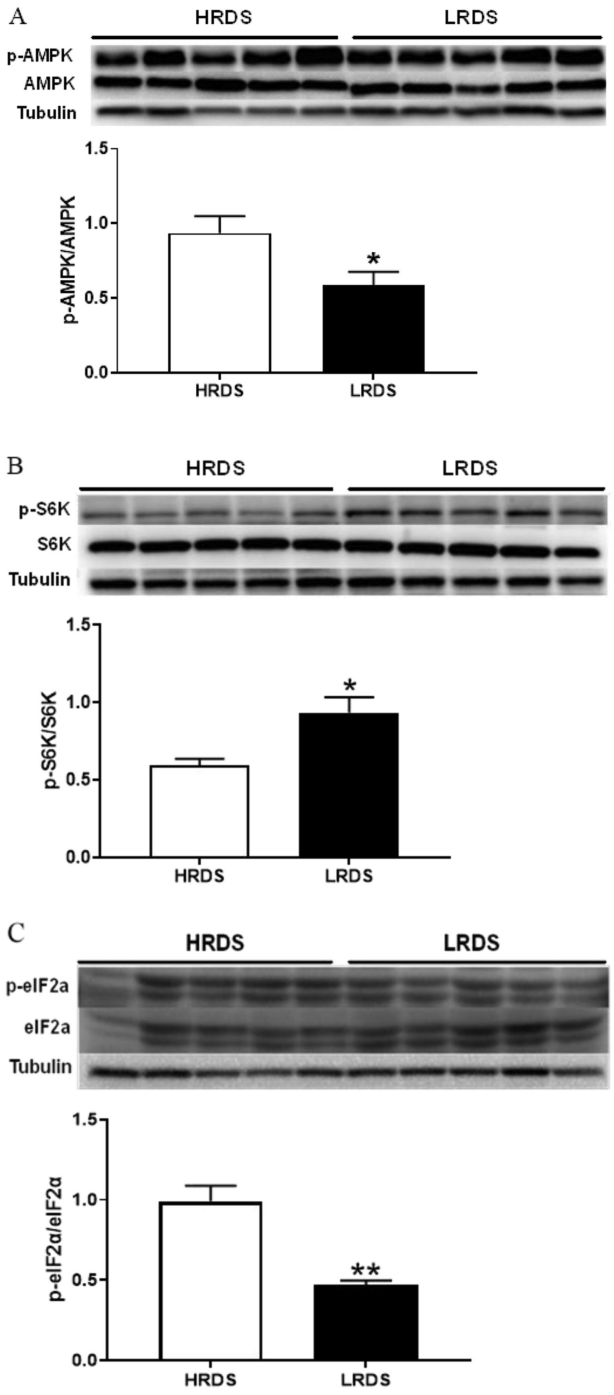

Fig. 4. Effects of rumen degradable starch (RDS) on protein expressions of AMPK, S6K and eIF2α in the biceps femoris (BF) muscle of growing goats. (A) The ratio of phosphorylated AMP-activated protein kinase to AMP-activated protein kinase (p-AMPK/AMPK); (B) the ratio of phosphorylated ribosomal protein S6 protein kinase to ribosomal protein S6 protein kinase (p-S6K/S6K); (C) the ratio of phosphorylated eukaryotic initiation factor α to eukaryotic initiation factor α (p-eIF2α/eIF2α). HRDS = high rumen degradable starch; LRDS = low rumen degradable starch. Results are shown as mean ± SEM, $n = 5$; *, $P < 0.05$; **, $P < 0.01$, compared with the HRDS.

3.7. Protein expression of signaling pathways involved in BF muscle protein synthesis

The phosphorylation state of AMPK, p70-S6 kinase (S6K) and eukaryotic initiation factor 2α (eIF2α) signaling proteins in the BF muscle are shown in Fig. 4. The decreased ratio of p-AMPK/AMPK

($P < 0.05$) and p-eIF2α/eIF2α ($P < 0.01$) was observed in the BF muscle in LRDS goats. Furthermore, LRDS significantly increased the ratio of p-S6K/S6K ($P < 0.05$) compared to the HRDS group.

4. Discussion

Since elevated dietary RDS levels due to higher grain processing increase the risk of subacute ruminal acidosis, and starch digestion in the small intestine provides 42% more energy compared with digestion in the rumen (Harmon, 2009; Ren et al., 2020), it is necessary to evaluate the influence of dietary RDS on animal health, feed efficiency, growth performance and carcass characteristics in growing goats before any suggestions can be asked about intensive livestock production. In the present study, we observed improvements in feed efficiency and growth performance in the LRDS group, indicating better energy utilization, which agrees with previous studies (Huntington et al., 2006; Harmon, 2009; Kazemi-Bonchenari et al., 2017; Shen et al., 2020). By limiting dietary grain processing, Owens et al. (1997) and Makizadeh et al. (2020) observed a greater feed efficiency in calves, which also supports our findings.

Ruminants have a high demand for glucose, so increasing the supply of glucose is beneficial for improving their growth and production performance (Nocek and Tamminga, 1991). In the present study, LRDS increased the concentration of plasma glucose in goats and did not affect the plasma insulin concentration, which was consistent with a previous study that showed that blood glucose concentration was greater for calves fed the diet containing steam-flaked corn grain than that containing ground corn grain (Makizadeh et al., 2020). Kazemi-Bonchenari et al. (2017) showed that reduction of dietary RDS content increased blood glucose concentration, but had no effect on the concentration of blood insulin. Both the absorption of exogenous glucose and endogenous liver gluconeogenesis contribute to blood glucose concentration (Nocek and Tamminga, 1991). Glucose and amino acids are mainly absorbed in the small intestine after feed digestion (Sala-Rabanal et al., 2018), withSGLT1 and glucose transporter 2 (GLUT2) both playing a key role in mediating the absorption of glucose in the small intestine (Lohrenz et al., 2011; Chen et al., 2016). In this study, we found higher mRNA expression of SGLT1 and GLUT2 in the jejunum in LRDS goats, which implied enhanced small intestinal starch digestion and small intestinal glucose transport in LRDS goats. No significant difference was observed in the gene expression of gluconeogenesis enzymes in the liver between the 2 groups. These results indicated higher postruminal starch digestion in LRDS goats, and the increased plasma glucose in LRDS goats mainly contributed to the absorption of exogenous glucose in the small intestine.

The supply of metabolizable protein (MP) to ruminants mainly comprises microbial (MCP) and feed origin protein, which pass from the rumen and are absorbed from the small intestine (Ipharraguerre et al., 2005). It has been documented that manipulating the ruminal fermentability of a diet affects rumen nitrogen metabolism and microbial protein synthesis (Clark et al., 1992; Dewhurst et al., 2000; Lu et al., 2019; Tosta et al., 2019). Previous studies showed that grain processing did not affect purine derivatives and MCP yield (Mutsvangwa et al., 2012; Brassard et al., 2015; Kazemi-Bonchenari et al., 2017). Instead, it has been shown that the forage:concentrate ratio rather than grain processing affected MCP synthesis and passage of MCP to the duodenum (Yang et al., 2000; Beauchemin et al., 2001; Yang and Beauchemin, 2004). Consistently, in our study, we found that the MCP synthesis was not affected by the reduction of RDS.

Besides, we observed an increased protein content of skeletal muscle in the LRDS group compared to the HRDS group, which was consistent with Jeyapalan et al. (2007). The transport and utilization of amino acids affect the development of skeletal muscle and protein synthesis (Sun et al., 2015). The estimated efficiency of MP and AA increased with abomasal infusion of glucose in dairy cows (Omphalius et al., 2020). Many others have found a suppressive effect of glucose on plasma AA concentration in lactating cows (Curtis et al., 2014; Nichols et al., 2016), and it has been suggested that absorption of AA by peripheral tissues such as skeletal muscle could account for their lower plasma concentration (Curtis et al., 2014; Omphalius et al., 2020). In the present study, LRDS reduced the concentration of total AA in plasma and tended to reduce BUN concentration, implying that increased glucose availability reduced the breakdown of amino acids and improved the balance of amino acids in LRDS goats. Contrary to previous studies (Nichols et al., 2016; Curtis et al., 2018; Omphalius et al., 2020), no significant changes in EAA concentration in plasma were observed between the 2 groups in this study. However, we found that LRDS decreased the concentration of Arg, His, Lys and Met in the plasma of goats, implying increased efficiency of use of EAA, which is consistent with Omphalius et al. (2020) and Curtis et al. (2018). LRDS also increased the concentration of EAA (particularly Arg) and free total AA and tended to increase the concentration of free BCAA in the BF muscle. These results indicated that the LRDS diet improved AA utilization for protein synthesis in the skeletal muscle of growing goats.

Amino acid transporters mediate the transmembrane transport of amino acids for the supply of nutrients to tissues (Taylor, 2014). LAT1 belongs to system L transporter family and transports large neutral amino acids such as BCAA (Yan et al., 2019). LAT1 performs an amino acid transport function by binding to the cell surface antigen 4F2hc (also known as SLC3A2) on the cell membrane to form a heterodimer (Javed and Fairweather, 2019), and is related to cell growth and proliferation (Zhang et al., 2020b). In this study, LRDS significantly increased the gene expression of both LAT1 and 4F2hc in the BF muscle of goats compared to the HRDS group, which was consistent with the increasing trend of free BCAA concentration in the BF muscle of LRDS goats. The increased gene expression of LAT1 and 4F2hc may provide more amino acids as anabolic signals and substrates for protein synthesis in the BF muscle (Drummond et al., 2010).

Glucose uptake in tissues is mediated by INSR and GLUT4 (Jaakson et al., 2018). GLUT4 is highly expressed in the BF muscle, cardiomyocytes and adipose tissue, and is involved in blood glucose homeostasis (Huang and Czech, 2007). The binding of insulin to its receptor (INSR) promotes the expression and translocation of GLUT4 through the PI3K/AKT/mTOR pathway, which leads to the uptake of glucose from fat and muscle tissue (Jaakson et al., 2018; Ueda-Wakagi et al., 2019). In our study, the gene expression of INSR and GLUT4 in the BF muscle of LRDS goats was increased, compared to the HRDS group, indicating a stronger glucose uptake capacity of skeletal muscle.

The availability of glucose is a prerequisite for translation (Zhang et al., 2020a). It is well known that AMPK and mTOR, which sense intracellular energy and nutrient availability, are the primary internal regulators of cell growth and proliferation (Gonzalez et al., 2020). Glucose can inhibit AMPK activity directly or by reducing the ratio of AMP/ATP indirectly (Lin and Hardie, 2018). By contrast, glucose starvation activates AMPK, thereby shutting down the mTOR1 pathway, which regulates the initiation and elongation steps of translation and further cell growth (Yamamoto et al., 2017; Lin and Hardie, 2018). The main role of mTOR1 activation is to promote translation and protein synthesis by activating 2 downstream target proteins S6K and 4E binding protein 1 (4EBP1) (Saxton and Sabatini, 2017; Guo et al., 2018). As eIF2α kinase, the general control non-inhibitory 2 (GCN2) is another signaling

pathway that senses cellular amino acid changes and regulates protein translation in eukaryotic cells (Pathak et al., 2019). Uncharged tRNA could activate GCN2, leading to phosphorylation of downstream eIF2α and inhibition of translation and protein synthesis (Dong et al., 2000). mTOR1 and GCN2 orchestrate cellular adaptation to amino acid levels (Ye et al., 2015). In our study, we found that phosphorylation of the AMPK protein was inhibited in the BF muscle of LRDS goats compared to the HRDS group, which was consistent with the results of other studies that found that glucose increases cellular energy levels and inhibits AMPK activity (Hardie et al., 2012; Lin and Hardie, 2018). Moreover, higher S6K phosphorylation was also observed in LRDS goats compared to the HRDS group in our study, indicating stronger mTOR1 activity and higher activation levels of the mTOR1-S6K pathway, and higher protein synthesis efficiency (Magnuson et al., 2012). In contrast, the phosphorylation of eIF2α was inhibited in the BF muscle of LRDS goats compared to the HRDS group. These results indicated that the LRDS diet increased plasma glucose availability, resulting in lower muscle catabolism and enhanced anabolism such as protein synthesis.

In addition, stronger mTOR1 activity may have contributed to the increased gene expression of LAT1 in this study. Several studies suggested that nutrient transporters may be downstream targets of mTOR (Liu et al., 2004; Roos et al., 2007; Rosario et al., 2013, 2016). Roos et al. (2007) demonstrated that placental mTOR regulates the activity of LAT1, but not system A transporters. Consistently, Rosario et al. (2016) showed that mTORC1 activation increased LAT1 activity and expression. Since intracellular BCAAs are potent activators of the mTOR signaling system, the regulation of LAT1 by mTOR represents a powerful cellular response mechanism to nutrient availability.

5. Conclusion

In summary, the results of the present study showed that reducing the content of dietary RDS enhanced postruminal starch digestion and increased plasma glucose, thereby promoting protein synthesis in the skeletal muscle of goats via the AMPK-mTOR pathway. These changes may contribute to improvements in growth performance and carcass traits in LRDS goats. Our findings highlight the regulation of dietary RDS content in the growth of goats and provide new insights into nutrient interactions coordinated by the AMPK-mTOR pathway in the skeletal muscle of ruminants.

Author contributions

Junhu Yao and **Chunjia Jin** conceived and designed the study; **Hanxun Bai**, **Gaofeng Liang**, **Ziqi Liang**, **Xiaodong Su** and **Dangdang Wang** conducted the research; **Ziqi Liang** analyzed and interpreted the data; and **Ziqi Liang** and **Chunjia Jin** wrote the manuscript; **Junhu Yao** revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix Supplementary data

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