Effects of γ-aminobutyric acid on feed intake, growth performance and expression of related genes in growing lambs

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This study was conducted to investigate the effects of rumen-protected γ-aminobutyric acid (GABA) on feed intake, growth performance and expression of related genes in growing lambs. A total of 24 lambs weaned at age of 50 days were divided into four block of six based on their BW, six lambs within a block were allocated to three pairs, which were then assigned randomly to three treatments with addition of rumen-protected GABA at levels of 0, 70 or 140 mg/day for 6 weeks. Dry matter intake was recorded weekly in three consecutive days, and BW was recorded every two weeks. At the end of the trial, four lambs from each group were slaughtered, and duodenum and ileum mucosa were obtained for measurement of mRNA abundance of GABA receptor and cholecystokinin receptor. Dry matter intake was higher ($P < 0.01$) in the lambs fed 140 mg/day GABA than that in the

three pairs, which were then assigned randomly to three treatments with addition of rumen-protected GABA at levels of 0, 70 and 140 mg/day, respectively. The rumen-protected GABA was used because the unprotected GABA was quickly degraded within the rumen (Wang *et al*., 2010) and non-protected GABA had little effect on feed intake and lactation performance in transition cows (Wang *et al*., 2013). The procedure for preparation of the rumen-protected GABA was described elsewhere (Wang *et al*., 2010). Each pen of two lambs was kept in an individual pen (2.5×2 m) and bedded on steel frame during the feeding trial lasting for 6 weeks. All the lambs were fed a total mixed ration (TMR) twice a day at 0700 and 1530 h, and had free access to drinking water. In brief, the TMR was prepared by cutting and blending the individual feed ingredients listed in Table 1, and then fed to the sheep in each pen. The daily amount of GABA was mixed with 1 g of corn and fed in two equal portions by top-dress feeding to individual sheep, to ensure that both lambs in the pen consumed the correct dose of GABA.

Sampling procedures and measurements

DMI and ADG. The amount of feed given was calculated to allow 10% orts. Feed offered and refused were recorded for three consecutive days (2^{nd}, 3^{rd} and 4^{th} days) weekly, to determine DMI. Nutrients compositions are listed in Table 1. The lambs were weighed on days 0, 14, 28 and 42 of the trial. Data were used to calculate ADG (mean of ADG for the period of days 0–14, 14–28 and 28–42). All measurements were conducted at 0630 h before feeding.

Composition of the TMR and feces. The TMR samples were collected from each feeding on 2^{nd}, 3^{rd} and 4^{th} days weekly. The samples were dried in an air-forced oven at 60°C for 48 h and then ground for later analysis of dry matter (DM, Association of Official Analytical Chemists, 1997, method 930.15) and CP (Association of Official Analytical Chemists, 1997, method 928.08). Contents of NDF and ADF were expressed inclusive of residual ash (Van Soest *et al*., 1991; Association of Official Analytical Chemists, 1997, method 973.18). Apparent digestibilities of nutrients were estimated using acid insoluble ash (AIA) as an internal marker based on the concentration of AIA in the diet and feces, where AIA was determined. Fecal samples of each pen were collected on the 4^{th} day biweekly, and then composited to be analyzed for chemical composition.

Table 1 *Ingredients and composition of the experimental basal diet*

Ingredients	DM basis (g/kg)
Bean curd residue	319
Distillers grains	223
Corn silage	110
Soybean straw	99.8
Corn	82.4
Wheat bran	73.4
Cottonseed meal	34.1
Barley	27.6
Premix[1]	21.0
Calcium carbonate	3.4
Salt	3.4
Calcium bicarbonate	2.2
Nutrient composition (g/kg DM)	
NDF	351
ADF	193
CP	162
AIA	16

DM = dry matter; AIA = acid insoluble ash.
[1]Formulated to provide (per kilogram of DM): Co, 200 mg; Cu, 15 000 mg; I, 600 mg; Fe, 8000 mg; Mg, 100 000 mg; Mn, 30 000 mg; Se, 200 mg; Zn, 40 000 mg; vitamin A, 4 000 000 IU; vitamin D, 400 000 IU; vitamin E, 8000 IU.

Serum variables. Blood samples were collected from the neck vein at 1100 h on days 14, 28 and 42 of the trial period, respectively, and then centrifuged at $3000 \times \mathbf{g}$ for 10 min to collect serum. Serum samples were stored at −20°C for later analysis of contents of GABA and CCK as the methods described elsewhere (Wang *et al*., 2013).

Slaughter trial and sampling

One animal of each pen was electrically stunned and slaughtered. Intestinal sections (3 cm for each section) from the duodenum and ileum were washed with double distilled water. The epithelium was separated from the underlying muscle layers and stored in liquid nitrogen until analyzed.

RNA extraction and determination of mRNA abundance

Total RNA was isolated from duodenum and ileum mucosa according to TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA). Reverse transcription reactions (20 μl) consisted of 16 μl total RNA, 1 μl of RNAse inhibitor (Promega, Madison, WI, USA), 1 μl dNTPs (Sigma, St. Louis, MO, USA) and 2 μl of $5 \times$ M-MLV RT reaction buffer (Promega) were used to get cDNA. The optimal reverse transcription procedure was 20°C for 5 min, followed by 42°C for 60 min and then 70°C for 5 min. Reactions were immediately stopped by putting reaction system on ice. The cDNA was stored at −20°C for real-time PCR.

Real-time PCR was performed with ABI PRISM 7700 sequence detection system (ABI Biosystems, Foster City, CA, USA) according to optimized PCR protocols. A total volume of 20 μl PCR reaction system was composited by 10 μl SYBR Green PCR Master Mix, 2.0 μl primer (1.0 μl forward and 1.0 μl reverse), 2.0 μl cDNA template and 6 μl sterile super-stilled water. The following protocol was employed for the PCR reaction: denature at 95°C for 3 min, 42 cycles of amplification (94°C for 30 s, 51°C for 30 s, 72°C for 60 s) and finally extension at 72°C for 7 min. The C_t value was recorded and the relative expression level ($2^{-\Delta\Delta C_t}$) was calculated for determination of related mRNA abundance. Primers used in the current study are given in Supplementary Table S1.

Statistical analysis

All data were analyzed using repeated measures by PROC MIXED model (SAS Release 6.12, 1988). Treatment, pen

Table 2 *Effects of dietary rumen-protected γ-aminobutyric acid (GABA) on dry matter intake (DMI), average daily weight gain (ADG), feed efficiency and nutrients digestibility in growing lambs*

	Rumen-protected GABA (mg/day)				*P* value	
Item	0	70	140	s.e.m.	Linear	Quadratic
DMI (g/day)	1146	1172	1248	14.2	<0.01	0.15
ADG (g/day)	284	282	310	15.6	0.22	0.45
Feed efficiency[1]	0.25	0.24	0.25	0.011	0.95	0.65
Digestibility (%)						
Dry matter	67.7	67.5	66.4	1.47	0.54	0.80
CP	63.3	63.5	64.5	1.53	0.65	0.85
NDF	55.1	54.6	55.0	1.47	0.99	0.81
ADF	38.7	37.6	37.4	0.91	0.41	0.76

[1]Feed efficiency = ADG/DMI.

Table 3 *Effects of dietary rumen-protected γ-aminobutyric acid (GABA) on the mRNA abundances of cholecystokinin (CCK), its receptors and GABA receptors in epithelium of duodenum and ileum in growing lambs*

	Rumen-protected GABA (mg/day)				*P* value	
Item	0	70	140	s.e.m.	Linear	Quadratic
Serum content						
CCK (pg/ml)	684^{A}	650^{A}	412^{B}	25.1	< 0.01	< 0.01
GABA (mmol/l)	27.7	28.8	28.6	0.74	0.33	0.46
Gene expression						
CCK						
In duodenum	0.27	0.24	0.30	0.076	0.80	0.64
In ileum	0.28	0.24	0.25	0.062	0.39	0.93
CCK-1 receptor						
In duodenum	0.18	0.16	0.21	0.045	0.38	0.40
In ileum	0.30	0.27	0.29	0.052	0.91	0.69
CCK-2 receptor						
In duodenum	2.03^{A}	1.07^{AB}	0.77^{B}	0.161	< 0.01	0.15
In ileum	1.15	0.99	0.93	0.208	0.72	0.72
GABA-A receptor						
In duodenum	1.40	1.55	1.18	0.143	0.33	0.19
In ileum	1.10	0.97	0.95	0.198	0.96	0.82
GABA-B receptor						
In duodenum	0.46^{B}	0.43^{B}	0.94^{A}	0.051	< 0.01	0.01
In ileum	0.54	0.45	0.56	0.138	0.56	0.55

[A,B]Values with different superscripts are significantly different ($P < 0.01$).

(sheep for variable at slaughter) within treatment, block and sampling time were included in the model as main effects and treatment × time was included as the interaction. Linear and quadratic effects of treatment were tested for DMI, ADG, serum contents of GABA and CCK, and gene expression related with GABA and CCK using orthogonal polynomial contrasts, respectively. Data are presented as the least squares mean, and differences were considered significant at $P < 0.05$.

Results and discussion

Addition of GABA increased DMI of the lambs linearly ($P < 0.01$, Table 2). The ADG and feed conversion efficiency did not differ among treatments ($P > 0.05$), though the lambs fed 140 mg/day rumen-protected GABA grew faster (numerically, by 9.1%) than others. We acknowledge that low replication may account for insufficient power to detect differences in growth rate.

Dietary GABA had no effect on digestibility of DM, CP, NDF and ADF ($P > 0.05$, Table 2). The rumen degradation rate of rumen-protected GABA used in the current study was about 15% (Wang *et al.*, 2010). Thus, the released GABA in rumen may have been about 10.5 or 21 mg/day in lambs fed 70 or 140 mg/day rumen-protected GABA, respectively. Unprotected GABA had limited effects on rumen pH, volatile fatty acids (acetate, propionate and butyrate) and ammonia-nitrogen concentrations (unpublished). Thus, dietary rumen-protected GABA did not exert significant effect on nutrients digestibility.

Serum CCK content decreased (linear, $P < 0.01$ and quadratic, $P < 0.01$), while serum GABA was not affected by GABA ($P >$