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Effects of rumen-protected γ-aminobutyric acid on feed intake, lactation performance, and antioxidative status in early lactating dairy cows

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ABSTRACT

The objective of this study was to investigate effects of rumen-protected γ-aminobutyric acid (GABA) on dry matter intake, milk performance, and serum metabolites in Chinese Holstein lactating cows. Thirty-nine multiparous cows were blocked based on days in milk (60 ± 6.3 d; mean ± SD) and milk production (30.9 ± 4.17 kg; mean ± SD), and were randomly assigned to 1 of 4 treatments, with rumen-protected GABA added at levels of 0, 0.8, 1.6, or 2.4 g/d, the actual predicted available amounts being 0, 0.30, 0.61, or 0.91 g of GABA/d, respectively. The experiment lasted for 8 wk, with the first week for adaptation. Milk yield and milk compositions were recorded weekly, and serum concentrations of GABA, neuropeptide Y, and biochemical and antioxidant variables were analyzed in the first, fourth, and seventh weeks of the study. Dry matter intake linearly increased in cows receiving added GABA compared with that for the control. Addition of 0.8 g of GABA/d was associated with higher milk yield than the other treatments, but contents of milk protein and fat did not differ across the treatments. Dietary GABA tended to quadratically enhance the serum content of GABA (23.6, 30.2, 29.8, or 28.3 mmol/L for 0, 0.8, 1.6, or 2.4 g/d, respectively), and increased neuropeptide Y, with the highest value (3.07 ng/L) for 0.8 g of GABA/d. Nonesterified fatty acid quadratically decreased with GABA addition, with the lowest value (218.1 μmol/L) for 0.8 g of GABA/d. Serum glutathione peroxidase and superoxide dismutase quadratically increased in cows fed GABA, whereas serum malondialdehyde was quadratically reduced for all GABA groups. Rumen-protected GABA quadratically improved N efficiency across all treatments, contributing to the enhanced production of milk and milk protein and reduced N emission to the environment. In conclusion, addition of rumen-protected GABA is beneficial for early lactation dairy cows in terms of feed intake, lactation performance, and animal health.
Key words: γ-aminobutyric acid, dry matter intake, milk performance, early lactation cow

INTRODUCTION

Feed intake by dairy cattle does not increase as rapidly as the increase in milk production in the early lactation stage. Therefore, lactating cows enter a period of negative energy balance. The mobilization of body reserves provides the enough energy needed for lactation, but changes the metabolic and endocrine status of dairy cattle (Veerkamp et al., 2003; Wang et al., 2010c), which may result in nutritional disorders such as fatty liver (Herdt, 2000). Cows should be supplemented with energy enough to achieve their genetic milk potential and to reduce the mobilization of body reserves in the early lactation stage (Pedernera et al., 2008; Wang et al., 2010c).

γ-Aminobutyric acid (**GABA**) is a functional NPN that is widely distributed in the central nervous system, peripheral nerve tissue, and nonneural tissue. It is synthesized mainly from glutamate through α-decarboxylation (Martin and Rimvall, 1993) and plays an important role in the hypothalamus of animals, regulating their feeding behavior. Seoane et al. (1984) reported that injecting a dose of 160 nmol of muscimol (a GABA-A receptor agonist) into the lateral ventricles increased the feed intake of satiated sheep, indicating that neuronal sensitivity to GABA is possibly related to the control of feeding behavior in ruminant animals. Injecting muscimol and baclofen (a GABA-B receptor agonist) into the nucleus accumbens centrum increased feed intake in engorged rats (Stratford and Kelley, 1999). In the hypothalamus arcuate nucleus, GABA is co-expressed with neuropeptide Y (**NPY**) for the regulation of feed intake in rats. Both GABA and NPY have partially shared similar signal transduction pathways (Pu et al., 1999). A positive effect of GABA-

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B has been observed on gastric acid secretion in *SSTR2* gene-deficient adult male mice (Piqueras and Vicente, 2004).

At present, GABA is not approved as a legal type of feed additive for livestock animals in China, but it has been approved as a kind of legal food additive for human use (MOH, 2011). It is demonstrated that feeding GABA could increase feed intake and weight gain in growing pigs (Fan et al., 2007) and weanling piglets (Yang et al., 2009), and decrease weight loss in lactating sows (Liang et al., 2009). However, information is limited with regard to the effects of GABA on the performance of lactating cows. We hypothesized that dietary supplementation of GABA could enhance DMI and milk production in early lactating cows. Thus, the objective of this study was to determine the effects of GABA addition on feed intake, milk performance, serum metabolic variables, and antioxidative status in the early lactation dairy cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

Thirty-nine multiparous Chinese Holstein dairy cows in early lactation (DIM = 68 ± 6.3, milk yield = 30.9 ± 4.17 kg/d; mean ± SD) were blocked into 10 groups based on DIM and milk production. Four cows were included in 9 blocks each, whereas the last block had 3 cows. The cows were randomly assigned within block to 1 of 4 dietary treatments: 0 (control), 0.8, 1.6, and 2.4 g of rumen-protected GABA/d. According to a previous study (Wang et al., 2010b), when GABA was mixed with an equal amount of solid palm oil as a coating and processed under air pressure of 9 kPa, the product had optimal protection from rumen degradation and maximal postruminal dissolution in vitro. The rumen-protected GABA used in the current study, containing 50% of GABA, was estimated to be 82.0% rumen protected, and the postruminal dissolution rate (Rossi et al., 2003) was 92.4% after in vitro incubation for 12 h (Wang et al., 2010b). It can be estimated that 1 g of rumen-protected GABA could provide 0.379 g of available GABA. Thus, the actually predicted amount of GABA was 0, 0.30, 0.61, or 0.91 g/d for 0, 0.8, 1.6, and 2.4 g of GABA/d, respectively. The rumen-protected GABA was mixed with the concentrate mixture to achieve the desired concentration. Cows were housed in individual tiestalls with free access to water and fed and milked at 0600, 1400, and 2000 h daily.

Ingredients of the experimental diet and nutrient content of the feed components are presented in Tables 1 and 2, respectively. Feed was offered 3 times per day. At each feeding time, distillers dried grains with solubles, alfalfa pellets, and apple pomace were mixed and offered first, and then corn silage was fed to the cows. Afterward, the concentrates with GABA and finally grass hay was offered ad libitum. The cows consumed all the feed offered except for grass hay. The residue of hay was recorded daily.

Table 1. Ingredients (DM basis) of basal diets used in the experiment

Ingredient	Amount, kg/d per cow
Ground corn	5.26
Soybean meal	1.05
Whole cottonseed	0.53
Cottonseed meal	0.74
Sesame meal	0.84
Wheat bran	0.84
Dicalcium phosphorus	0.21
Limestone	0.19
Sodium bicarbonate	0.14
Salt	0.1
Alfalfa pellet	2.5
Corn silage	3.8
Apple pomace	0.6
DGGS[1]	1.9
Premix[2]	0.1
Grass hay[3]	Ad libitum

[1]Distillers dried grains with solubles.

[2]Provided per kilogram of premix: 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1,250 IU of vitamin E, 14,000 mg of Zn, 100 mg of Se, 180 mg of I, 3,000 mg of Fe, 40 mg of Co, 3,000 mg of Mn, and 3,000 mg of Cu.

[3]Grass hay was fed at approximately 4 kg/d per cow (DM basis).

Sampling, Measurement, and Analyses

Feed offered and orts were weighed for 2 consecutive days in the first, fourth, and seventh weeks to determine feed intake. During the sampling periods, proportional amounts of each feed offered was collected, pooled according to treatment, and sampled for chemical analysis. All samples were dried in a forced-air oven at 60°C for 48 h, and placed in sealed containers until analysis. Samples were ground to pass a 2-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA) and then through a 1-mm screen in a Cyclotec mill (Tecator 1093; Tecator AB, Höganäs, Sweden). Feed samples were analyzed for DM, CP (AOAC International, 1997; method 988.05), NDF (Van Soest et al., 1991), and ADF (AOAC International, 1997; method 973.18).

Milk yield was measured weekly on the fourth day, and milk samples were collected from each milking using Waikato Milking System meters (Waikato Milking Systems NZ Ltd., Hamilton, New Zealand). Milk samples were composited in a proportion of 4:3:3 from each milking. A 50-mL subsample was treated with bronopol (milk preservative; D & F Control Systems Inc., San Ramon, CA) and stored at 4°C for later analysis

Table 2. Composition (mean ± SEM) of feed components used in the experimental diets

Composition	Concentrate mixture	DDGS[1]	Alfalfa pellet	Grass hay	Apple pomace	Corn silage
DM, %	88.0 ± 2.16	90.0 ± 2.77	92.0 ± 2.77	91.6 ± 1.71	90.1 ± 2.62	19.8 ± 2.45
CP, % of DM	17.0 ± 1.46	28.3 ± 0.86	17.8 ± 1.43	10.8 ± 0.92	9.3 ± 0.87	8.1 ± 1.57
NDF, % of DM	16.5 ± 2.04	39.6 ± 1.66	40.0 ± 2.95	69.1 ± 1.80	48.9 ± 1.23	65.2 ± 4.80
ADF, % of DM	8.4 ± 0.62	46.2 ± 1.64	33.2 ± 1.05	39.5 ± 2.21	30.0 ± 0.88	38.4 ± 1.97
NE_L,[2] MJ/kg of DM	7.03	7.95	5.94	4.35	6.19	6.07

[1]Dried distillers grains with solubles.

[2]Calculated based on Ministry of Agriculture individual feedstuffs recommendations (MOA, 2004).

of fat, true protein, and lactose by infrared analysis (MilkoScan; Foss Electric A/S, Hillerød, Denmark; Laporte and Paquin, 1999). Another subsample without bronopol was used to determine the concentration of MUN using the diacetyl monoxime-binding assay described by Wang et al. (2010a).

Blood samples (10 mL) were collected from the coccygeal vein by syringe approximately 5 h after feeding on the first day of the first, fourth, and seventh week, immediately placed into a 10-mL tube, allowed to clot, and then centrifuged at 3,000 × *g* for 10 min at 4°C to collect serum. The serum was frozen at −20°C until later quantification of NEFA and glucose (McCutcheon and Bauman, 1986), BUN (Rahmatullah and Boyde, 1980), malondialdehyde (**MDA**), glutathione peroxidase (**GSH-Px**) activity, and superoxide dismutase (**SOD**; Zhang et al., 2006). The GABA was measured by HPLC according to the procedure described by Ebert et al. (1997). Briefly, the serum was mixed with equal amount of perchloric acid and centrifuged at 8,000 × *g* for 10 min at 4°C to remove precipitated proteins. Then, the supernatant was filtered to remove remaining high-molecular-weight compounds. The *ortho*-phthalaldehyde derivative of GABA was prepared immediately before sample injection. The determination of NPY basically followed the protocol by McShane et al. (1992). In brief, a 200-μL aliquot of serum was pipetted into tubes containing assay buffer. Anti-neuropeptide Y (200 μL) was added to all tubes except total-count tubes and nonspecific binding tubes, and then incubated for 24 h at 4°C. Monoiodinated [^{125}I] porcine NPY (200 μL) was added to all tubes and then allowed to incubate for 24 h at 4°C. A preprecipitated sheep anti-rabbit second antibody was added and incubated for 15 min at room temperature. The PBS was added followed by centrifugation at 3,000 × *g* for 10 min at 4°C , and then the supernatant was removed and pellets were counted in a gamma counter.

Calculations and Statistical Analysis

Intake of NE_L was calculated as the sum of the net energy content of individual feed and their proportion in the ration multiplied by DMI. All the data were analyzed using the mixed model procedure in SAS statistical software (SAS Institute, 2000) using the covariance type AR (1) for repeated measures. A randomized incomplete block design with repeated measurements was used, with week, treatment, treatment × week, and block as the main effects. Cow was the subject for the repeated measures. Results are reported as least squares means. Linear and quadratic effects of treatment were tested for milk yield, milk protein yield, milk composition and serum variables using orthogonal polynomial contrasts. For regression analysis, PROC REG was applied to determine the DMI or milk yield relative to GABA doses. Probability values of $P < 0.05$ were used to define statistical significance, and values $P < 0.10$ and $P > 0.05$ were accepted as statistical trends.

RESULTS

Feed Intake

The result of intake influenced by addition of rumen-protected GABA is shown in Table 3. The intake of grass hay increased in cattle fed GABA compared with that of the control ($P = 0.02$), resulting in increased total DMI and NE_L intake ($P = 0.02$), with little difference among the different amounts of GABA. Change in DMI with experimental period is shown in Figure 1. The DMI in the control cows without GABA did not change throughout the experiment, but the cows receiving added GABA indicated higher DMI in the fourth and seventh week compared with the control.

Lactation Performance

Milk yield was higher in cattle fed 0.8 g of GABA/d than that of the control and of the cows fed 2.4 g of GABA/d throughout the experiment (Figure 1). Addition of GABA had a quadratic effect on milk yield ($P < 0.01$; Table 3). However, no difference was found between the control and cows fed 1.6 or 2.4 g of GABA/d and between those fed 0.8 and 1.6 g of GABA/d. A second-degree curve was fitted to present the changes

Table 3. Effects of dietary addition of rumen-protected γ-aminobutyric acid (GABA) on feed intake and milk production in early lactating dairy cows

	Rumen-protected GABA, g/d					*P*-value	
Item	0	0.8	1.6	2.4	SEM	Linear	Quadratic
DMI, kg/d							
Grass hay	2.1	2.9	2.9	3.0	0.24	0.02	0.13
Total	20.9	21.7	21.7	21.8	0.24	0.02	0.13
NE_L intake, MJ/d	136.0	139.6	139.7	140.1	1.06	0.02	0.13
Milk yield, kg/d	30.0	32.0	30.8	30.0	0.37	0.47	<0.01
Milk protein yield, kg/d	0.90	0.98	0.96	0.93	0.014	0.39	<0.01
Milk composition, %							
Protein	3.08	3.02	3.13	3.11	0.041	0.27	0.71
Fat	3.63	3.55	3.60	3.51	0.041	0.11	0.79
Lactose	4.96	5.01	5.00	5.05	0.022	0.01	0.99
Urea nitrogen, mg/dL							
In milk	14.8	14.9	14.6	14.4	0.40	0.40	0.68
In serum	7.9	7.9	7.3	7.5	0.21	0.21	0.87
Nitrogen efficiency[1]	0.26	0.28	0.28	0.27	0.006	0.48	0.02

[1]Ratio of milk protein yield to CP intake.

in milk yield (Y, kg/d) with the added level of GABA (X, g/d): $Y = -1.0938X^2 + 2.475X + 30.18$ ($R^2 = 0.76$; $P = 0.04$). From the equation, it may be estimated that the optimized GABA level is at 0.9 g/d for milk production. Contents of milk fat, milk protein, and MUN were not different among the 4 treatments. All the cows fed GABA had higher lactose contents than those of the control ($P = 0.01$). Nitrogen efficiency quadratically increased in cows receiving 0.8 and 1.6 g of GABA/d compared with the control cows ($P = 0.02$; Table 3).

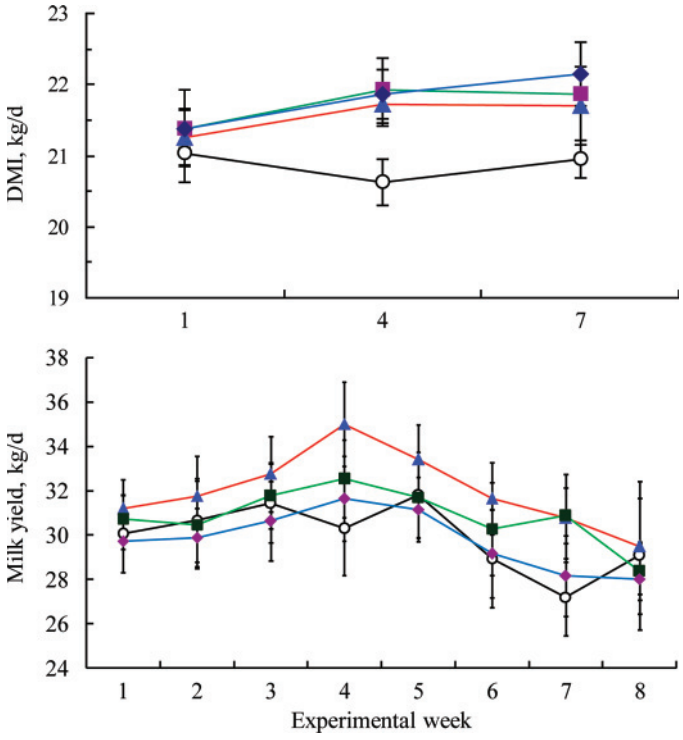

Figure 1. Change in DMI (A) and milk yield (B) with experimental periods for the control cows (○), and cows fed 0.8 (▲), 1.6 (■), and 2.4 g of rumen-protected γ-aminobutyric acid/d (◆). Bars indicate the standard error. Pooled SEM = 0.24. Color version available in the online PDF.

Metabolites

The results of treatment effects on serum variables are presented in Table 4. The serum concentration of GABA tended to quadratically increase in the GABA-added cows compared with the control ($P = 0.06$), with no difference among the 3 GABA-added treatments. The cows added with 0.8 or 1.6 g of GABA/d had quadratically higher NPY concentration ($P = 0.05$) compared with the others. Serum glucose was not altered among all treatments. Serum concentration of NEFA was highest in control cows (298.6 μmol/L) and decreased with GABA treatment (quadratic, $P = 0.04$; linear, $P = 0.08$), with the lowest concentration in cows with 0.8 g of GABA/d (218.1 μmol/L). For variables related to antioxidative status, serum SOD concentration increased linearly for all cows fed GABA ($P = 0.04$). Serum concentration of GSH-Px increased in cows fed GABA (quadratic, $P = 0.01$; linear, $P = 0.09$), with the highest value in cows fed 1.6 g of GABA/d (141.0 U/mL), whereas MDA was reduced for all groups fed GABA (quadratic, $P = 0.02$; linear, $P = 0.08$), with the lowest value in cows fed 1.6 g of GABA/d (2.08 mmol/mL). Serum SOD concentration increased linearly for all cows fed GABA ($P = 0.04$).

DISCUSSION

Injecting the GABA receptor agonist into the ventral tegmental area and the paraventricular nucleus

Table 4. Effects of dietary addition of rumen-protected γ aminobutyric acid (GABA) on serum metabolic variables in early lactating dairy cows

	Rumen-protected GABA, g/d					*P*-value	
Item[1]	0	0.8	1.6	2.4	SEM	Linear	Quadratic
GABA, mmol/L	23.6	30.2	29.8	28.3	2.09	0.16	0.06
NPY, ng/mL	2.82	3.07	3.05	2.62	0.165	0.43	0.05
Glucose, mmol/L	3.03	2.95	3.13	3.11	0.115	0.43	0.83
NEFA, μmol/L	298.6	218.1	230.0	237.1	20.59	0.08	0.04
SOD, U/mL	102.6	110.0	110.0	113.6	3.45	0.04	0.58
GSH-Px, U/mL	106.0	125.5	141.0	121.2	7.78	0.09	0.01
MDA, nmol/mL	2.72	2.32	2.08	2.39	0.145	0.08	0.02

[1]NPY = neuropeptide Y; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase activity; MDA = malondialdehyde.

increased animal feed intake (Echo et al., 2002; Stratford and Wirtshafter, 2004). Moreover, dietary GABA increased feed intake and daily weight gain in growing pigs (Fan et al., 2007) and weanling piglets (Yang et al., 2009). In the present study, the brain concentrations of GABA and NPY were not measured, whereas feeding GABA did induce a quadratic increase in the serum concentration of NPY ($P = 0.05$) and tended to enhance the GABA ($P = 0.06$), indicating that dietary GABA was absorbed. Neuropeptide Y has been considered to be an important feed intake regulator in mammalian animals (Pu et al., 1999); thus, enhanced feed intake in cows fed GABA is possibly associated with increased serum contents of GABA and NPY in these cows. However, the cows fed higher dose of GABA (2.4 g/d) kept higher DMI, but the serum NPY content was lower, indicating that other mechanisms than NPY-mediated process could be involved in regulation of intake.

Milk production and milk protein yield were increased quadratically with the increasing level of GABA (Table 3). Increased DMI from grass hay increased the net energy and CP intake, which is probably one factor that increased the milk yield and milk protein yield of cows. Increased yield of milk and protein did not correspond exactly with the increase in DMI with GABA supplementation. Quadratic effects were detected in milk and protein yields. Milk yield is positively correlated with serum prolactin (Erb et al., 1980) and GABA is a type of prolactin release-inhibiting factor (Hinuma et al., 1998). Thus, the decreased milk yield in cattle fed high levels of GABA may be mediated by decreased serum prolactin concentrations in dairy cattle. Nakayama et al. (2006) found that prolactin secretion could be inhibited by GABA-A receptor agonist or enhanced by GABA-C receptor agonist in rats. Little information exists on the effect of GABA on prolactin secretion in dairy cattle, which warrants further study.

Concentrations of NEFA were decreased in cows consuming GABA. The reduction in NEFA is beneficial for the health of the dairy cows in early lactation (Overton and Waldron, 2004). Increased DMI ($P = 0.02$) contributed to better antioxidative status for cows fed GABA, reflected by variation of serum metabolites. The GSH-Px converts H_2O_2 into less-dangerous reduced forms and SOD plays an important role in antioxidant defense mechanism in animals (Halliwell and Chirico, 1993), whereas MDA is one of the lipid peroxidation products and increased MDA content is considered to be an indicator of oxidative stress (Armstrong and Browne, 1994). In our study, the decreased MDA and increased GSH-Px and SOD indicated an enhanced antioxidative status in the cows fed GABA. Enhanced antioxidative status indicated by linearly increased contents of SOD ($P = 0.04$) and GSH-Px ($P = 0.09$), and linearly decreased MDA ($P = 0.08$) of cows fed GABA was consistent with decreased serum NEFA concentration (Table 4). A relationship likely exists between oxidative status and energy balance, as reported in other studies (Miller et al., 1993; Gabai et al., 2004).

CONCLUSIONS

Addition of rumen-protected GABA can linearly increase the intake of hay in early lactation dairy cows, resulting in increased DMI and NE_L intake. In the present study, the optimal addition level of rumen-protected GABA was 0.8 g/d, which was associated with increased nitrogen efficiency and milk and protein yields. We inferred that the dietary addition of rumen-protected GABA is beneficial for early lactating dairy cattle.

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