Effects of rumen-protected γ -aminobutyric acid on performance and nutrient digestibility in heat-stressed dairy cows

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ABSTRACT

This experiment was conducted to investigate the effects of rumen-protected γ -aminobutyric acid (GABA) on performance and nutrient digestibility in heat-stressed dairy cows. Sixty Holstein dairy cows (141 ± 15 d in milk, 35.9 ± 4.3 kg of milk/d, and parity 2.0 ± 1.1) were randomly assigned to 1 of 4 treatments according to a completely randomized block design. Treatments consisted of 0 (control), 40, 80, or 120 mg of true GABA/kg of dry matter (DM). The trial lasted 10 wk. The average temperature-humidity indices at 0700, 1400, and 2200 h were 78.4, 80.2, and 78.7, respectively. Rectal temperatures decreased linearly at 0700, 1400, and 2200 h with increasing GABA concentration. Supplementation of GABA had no effect on respiration rates at any time point. Dry matter intake, energy-corrected milk, 4% fat-corrected milk, and milk fat yield tended to increase linearly with increasing GABA concentration. Supplementation of GABA affected, in a quadratic manner, milk protein and lactose concentrations, and milk protein yield, and the peak values were reached at a dose of 40 mg of GABA/kg. Milk urea nitrogen concentration responded quadratically. Total solids content increased linearly with increasing GABA concentration. Supplementation of GABA had no effect on milk yield, lactose production, total solids, milk fat concentration, somatic cell score, or feed efficiency. Apparent total-tract digestibilities of DM, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber were similar among treatments. These results indicate that rumen-protected GABA supplementation to dairy cows can alleviate heat stress by reducing rectal temperature, increase DM intake and milk production, and improve milk composition. The appropriate supplemental GABA level for heat-stressed dairy cows is 40 mg/kg of DM.

Key words: γ -aminobutyric acid, milk yield, heat stress

INTRODUCTION

Heat stress has a variety of negative effects in the dairy industry. It can decrease feed intake (McGuire et al., 1989; Settivari et al., 2007; Rhoads et al., 2009), increase maintenance requirements (Huber, 1996; Fox and Tylutki, 1998) and diet digestibility (Ole Miaron and Christopherson, 1992), reduce rumen fermentation (Bernabucci et al., 2009) and milk yield (Collier et al., 1982; West, 2003; Rhoads et al., 2009), and compromise milk quality (Collier et al., 1982; West, 1999). Despite remarkable advances in the construction design of animal housing facilities and cooling technology (Armstrong, 1994; Burgos, et al., 2007), heat stress remains a costly issue for the dairy industry (St-Pierre et al., 2003). Consequently, it is clearly necessary to develop new strategies, based on physiological or metabolic adaptations, which can help maintain dairy cow health and milk yield during the warm summer months.

γ -Aminobutyric acid (**GABA**) is an inhibitory neurotransmitter in the adult mammalian central nervous system, the peripheral nervous system, and some non-neuronal tissues (Watanabe et al., 2002), and has certain physiological functions such as regulating body temperature and feed intake. γ -Aminobutyric acid participates in the neuronal network that downregulates body temperature in rats (Yakimova et al., 1996; Pierau et al., 1997) and rabbits (Frosini et al., 2000, 2003). Miyazawa et al. (2012) reported that a single oral administration of GABA induced a decrease in body core temperature in a hot environment. In addition, GABA increased feed intake in chicks (Jonaidi et al., 2002; Tajalli et al., 2006), and the intracerebroventricular injection of 2 types of GABA receptor agonists, GABA-A receptor agonist and GABA-B receptor agonist, stimulated feed intake in rats (Ebenezer, 1990; Pu et al., 1999). Furthermore, several publications have suggested that GABA can alleviate the negative effects of heat stress, resulting in a greater performance in chicks (Dai et al., 2011; Zhang et al., 2012) and pigs (Fan et al., 2007). Vaiva et al. (2004) reported that individuals with low plasma GABA levels

Received March 12, 2013.

Accepted May 13, 2014.

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are more vulnerable to stress-related disorders such as acute posttraumatic stress disorder. However, limited research has been done on GABA supplementation to dairy cows under heat stress. Therefore, the objective of this study was to investigate the effects of rumen-protected GABA on performance and nutrient digestibility in heat-stressed dairy cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

All animals involved in this study were cared for according to the principles of the Chinese Academy of Agricultural Sciences Animal Care and Use Committee (Beijing, China). Sixty lactating Holstein dairy cows (141 ± 15 DIM, 35.9 ± 4.3 kg of milk/d, and parity 2.0 ± 1.1) were blocked by parity (primiparous or multiparous), average daily milk yield, and DIM, and randomly assigned to 1 of 4 treatments ($n = 15$) according to a completely randomized block design. Treatments were 0, 40, 80, or 120 mg of GABA/kg of DM (control, **40GABA**, **80GABA**, or **120GABA**, respectively; supplemented as 0, 100, 200, or 300 mg of rumen-protected GABA product, respectively). Rumen-protected GABA, containing 40% GABA with 60% palm oil coating, was manufactured by the Chinese Academy of Agricultural Sciences.

The basal diet (as TMR) was formulated to meet or exceed the nutrient requirements of energy, protein, minerals, and vitamins according to the Feeding Standard of Dairy Cattle of China (China NY/t34, 2004). Diets (DM basis) contained 16.7% corn silage, 8.7% Chinese wild rye, 14% alfalfa hay, 23% corn, 9.4% barley, 7.4% soybean meal, 3.4% cottonseed meal, 3.7% dry distillers grains, 3.4% rapeseed meal, 8.3% cottonseed, 0.54% dicalcium phosphate, 0.49% salt, 0.64% sodium bicarbonate, and 0.33% vitamin-mineral premix. The TMR contained 16.9% CP, 39.1% NDF, 22.5% ADF, 1.07% Ca, and 0.48% P, with NE_L of 1.67 Mcal/kg of DM. All cows were individually fed a TMR 3 times daily (at 0600, 1400, and 2000 h) to ensure 10% refusals, and the GABA supplement was top-dressed on the TMR at the a.m. feeding. Orts were recorded before the a.m. feeding. During the experimental period, cows were housed in tie-stalls in a mechanically ventilated barn divided into 4 plots with 15 cows each and had free access to fresh water. The experimental period consisted of 2 wk for adaptation to the diet and 8 wk for sampling.

Sampling and Measurements

The daily DMI for individual cows was calculated by subtracting the Orts from the feed offered. Samples of

offered TMR were collected daily. Orts were sampled from each cow and composited for each treatment. Dry matter of weekly representative samples of TMR and Orts from each treatment was determined by oven drying at 65°C to a constant weight. Samples were ground to pass through a 50-mesh screen (297- μm opening) using a Cyclotec 1093 Mill (Tecator AB, Höganäs, Sweden) before analysis. The NDF and ADF were analyzed using procedure A of Van Soest et al. (1991). Sodium sulfite was not used in the procedure for NDF determination. A heat-stable α -amylase (A-3306; Sigma Chemical Co., St. Louis, MO) was used in the NDF analysis and NDF was expressed inclusive of residual ash. Nitrogen was analyzed using the macro-Kjeldahl nitrogen test (AOAC, 1990; method 960.52) with a Kjeltec System 1026 distilling unit (Tecator AB). Ash content was determined by incineration at 550°C overnight, and the OM content was calculated as the difference between 100 and the percentage of ash (AOAC, 1990; method 942.05). Ether extract content was determined using a Soxtec system HT6 apparatus (Tecator AB) according to AOAC (1990; method 920.39). Calcium content was determined by atomic absorption spectroscopy (model 5100; PerkinElmer Inc., Norwalk, CT) according to AOAC (1990; method 985.35). Phosphorus was determined by spectrophotometer (Spectronic 1001; Bausch & Lomb Inc., Rochester, NY) according to AOAC (1990; method 986.24). Composite TMR samples were analyzed for acid-insoluble ash (**AIA**; Van Keulen and Young, 1977) as a digestibility marker.

Cows were milked 3 times daily (at 0600, 1400, and 2000 h), with yields recorded at each milking. Milk samples from each cow were collected on d 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70. Milk aliquots were stored at 4°C until analysis. Fat, protein, lactose, TS, and MUN concentrations were determined by mid-infrared spectrophotometry using a MilkoScan analyzer (MilkoScan Type FT120; Foss Electric A/S, Hillerød, Denmark). Somatic cell counts were determined using a Fossomatic 5000 somatic cell counter (Foss Electric A/S). Individual SCC records were transformed to SCS (Shook, 2006) as follows: $\text{SCS} = \log_2[\text{SCC (cells per mL)} / 100,000] + 3$. Both 4% FCM and ECM were calculated using the following equations (Tyrrell and Reid, 1965): $4\% \text{ FCM} = 0.4 \times \text{milk (kg)} + 15 \times \text{fat (kg)}$ and $\text{ECM} = 0.327 \times \text{milk (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{protein (kg)}$. Feed efficiency was calculated as daily milk yield per kilogram of feed DMI on an individual-cow basis.

Fecal samples (400 g per sampling) were collected from the rectum or the ground, when fresh, at the end of the experimental period at 0900, 1500, and 2100 h (d 67); at 0300, 0600, 1200, and 1800 h (d 68); and at 0000 h (d 69). Samples were composited per animal on an as-is basis, dried at 65°C in a forced-air oven

to constant weight, and ground through a 1-mm sieve. Samples were analyzed for ash/OM, CP, NDF, ADF, ether extract, and AIA, as indicated above. Apparent total-tract digestibility was estimated, using AIA as an intrinsic digestibility marker (Foley et al., 2006).

Rectal temperatures were measured 3 times daily, at 0700, 1400, and 2200 h, using a glass mercury thermometer (Nasco, Ft. Atkinson, WI). Respiration rates were measured daily, at 0700, 1400, and 2200 h by counting the number of flank movements in 1 min. Air temperature and relative humidity were recorded daily (0700, 1400, and 2200 h) using measurement equipment (Beijing Yaguang Equipment Co. Ltd., Beijing, China). The equipment was placed in the center of each lot at a height of about 1.5 m from the ground. The temperature-humidity index (**THI**) was calculated using the following formula: $\text{THI} = \text{td} - (0.55 - 0.55 \text{ RH}) \times (\text{td} - 58)$, where td is the dry-bulb temperature in $^{\circ}\text{F}$ and RH is the relative humidity expressed as a percentage (NOAA, 1976).

The degradation rate of ruminally protected GABA in nylon bags (7 cm $\times$ 9 cm; pore size: 45 μm) was determined as described by Ørskov and McDonald (1979) using 3 rumen-cannulated lactating dairy cows. Duplicate bags containing approximately 5.0 g of ruminally protected GABA were incubated in the rumen of each animal for 2, 4, 6, 12, 24, 36, and 48 h. Nylon bags were introduced at the corresponding hours in the rumen and were removed from the rumen simultaneously, as suggested by Vanzant et al. (1998) to reduce the error. Bags were immediately washed with cold tap water until clear and then dried in a forced-air oven at 60 $^{\circ}\text{C}$ for 72 h. The concentration of GABA was determined by GC-MS/selected ion monitoring according to the methods of Suñol et al. (1988), with some modifications in sample preparation and derivatization procedures. The derivatization procedures were modified from the method of Jiang et al. (2004). Briefly, a GABA standard was purchased from Sigma Chemical Co. (purity of 99%). Aminovaleric acid (**AVA**) served as internal standard. Standard mixtures were prepared by adding different concentrations of unlabeled GABA to a fixed amount of AVA used for quantification of GABA. A 0.5-g sample of incubated ruminally protected GABA was dissolved in 500 mL of HCl (7.8 mol/L). After being filtered, 100 μL of supernatant was added to a centrifuge tube, and then 300 μL of deionized water was also added. After being vortexed (shaken vigorously) for 10 min, the mixed solution was centrifuged at 20,000 $\times g$ for 10 min at 4 $^{\circ}\text{C}$. Three hundred microliters of the supernatant and 100 μL of AVA (2 $\mu\text{g}/\text{mL}$) were added to a vial with a sealing cap and dried under N_2 , and then derivatized with 100 μL of bis(trimethylsilyl)trifluoroacetamide and 100 μL of

pyridine at 75 $^{\circ}\text{C}$ for 1 h. An Agilent gas chromatograph mass spectrometer (6890N-5975B; Agilent Technologies Inc., Santa Clara, CA) equipped with an HP-5MS column (30-m length, 0.25-mm i.d., and 0.25- μm film thickness; J&W Scientific Inc., Folsom, CA) was used. Hydrogen (99.999% purity) was used as the carrier gas with a flow of 1.0 mL/min. The oven temperature was programmed as follows: temperature was maintained at 70 $^{\circ}\text{C}$ for 1 min and then the temperature increased to 250 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C}/\text{min}$ and was maintained for 10 min. The electron impact was 70 eV and source temperature was 230 $^{\circ}\text{C}$. The quadrupole temperature was 150 $^{\circ}\text{C}$ and the interface temperature of GC-MS was 280 $^{\circ}\text{C}$. The solvent delay time was 7 min and the injection volume was 1 μL . The detection limit of GABA was 1 $\mu\text{mol}/\text{L}$ for each sample. The percentages of disappearance of ruminally protected GABA at each incubation time were calculated from the proportion remaining after incubation in the rumen using the equation of Sharma and Erdman (1988). Parameters of ruminal disappearance were computed according to the following nonlinear model (Ørskov and McDonald, 1979):

$$P = a + b \times (1 - \exp^{-ct}),$$

where P (%) denotes the proportion of the material degraded (lost from the bag) at time t (h); a (%) is the soluble, readily degradable fraction; b (%) is the insoluble, potentially degradable fraction; and c (%/h) is the degradation constant for fraction b . Effective degradability of DM (ED) from ruminally protected GABA was calculated using the following equation:

$$\text{ED} = a + [bc/(c + k)],$$

where a , b , and c are constants from the nonlinear model described above, and k is the ruminal passage rate, calculated at 6.1%/h for ruminally protected GABA, from the equations developed by the NRC (2001) for concentrates.

Blood samples were collected from all animals before the morning feeding via jugular venipuncture into heparinized tubes on d 42, and then centrifuged at 3,000 $\times g$ for 15 min at 4 $^{\circ}\text{C}$ to obtain plasma and stored at -80 $^{\circ}\text{C}$ for GABA analysis. Plasma GABA was determined by GC-MS/selected ion monitoring according to the methods of Suñol et al. (1988) with some modifications in sample preparation and derivatization procedures. A 100- μL plasma sample was added to a centrifuge tube, and then 200 μL of deionized water and 100 μL of HCl (7.8 mol/L) were also added. The content of GABA was determined by the Agilent gas chromatograph mass spectrometer as indicated above.

Table 1. Effects of rumen-protected γ -aminobutyric acid (GABA) on body temperature variables and blood GABA content in heat-stressed dairy cows

Item	Treatment ¹				SEM	P-value		
	Control	40GABA	80GABA	120GABA		Linear	Quadratic	Cubic
Rectal temperature (°C)								
0700 h	39.28	39.09	39.12	39.22	0.035	0.01	0.37	0.25
1400 h	39.71	39.53	39.44	39.60	0.036	0.01	0.15	0.68
2200 h	39.46	39.29	39.23	39.37	0.035	0.03	0.26	0.73
Respiration rate (breaths/min)								
0700 h	71.2	68.9	68.2	71.6	0.89	0.77	0.62	0.32
1400 h	75.3	74.7	73.9	75.7	1.31	0.62	0.35	0.88
2200 h	73.5	73.1	71.9	73.8	1.21	0.58	0.52	0.69
Plasma GABA (μ mol/L)	2.17	3.70	4.67	4.91	0.194	0.06	0.23	0.47

¹Cows were fed a basal diet (control) or basal diet supplemented with 40, 80, or 120 mg of GABA/kg of DM.

Statistical Analysis

Data for body temperature, DMI, milk yield, and milk composition were analyzed as a completely randomized block design with repeated measures using PROC MIXED of SAS (SAS Institute, 2005). The MIXED statistical model used for analysis was as follows:

$$Y_{ijk} = \mu + T_i + D_{j(i)} + W_k + (TW)_{ik} \\ \times (b + \Phi_j) \times X_{ij} + E_{ijk},$$

where Y_{ijk} = dependent variable measured at week k on the j th cow assigned to the i th treatment, μ = population mean, T_i = treatment effect, $D_{j(i)}$ = random effect of the j th cow within the i th treatment, W_k = time effect, $(TW)_{ik}$ = fixed interaction effect between treatment and time, b = the common regression coefficient of pretreatment data of X_{ij} , Φ_j = the slope deviation of the i th treatment from the common slope b , X_{ij} = the pretreatment data of cow j on treatment i , and E_{ijk} = random error associated with the j th cow assigned to the i th treatment at week k . Fixed effects in the model included treatment, time (hour of day for body temperature and week for DMI, milk yield, and milk composition), and treatment $\times$ time interaction. The variance for each cow was used as random effect. The mean daily body temperature, DMI, milk yield, and milk composition measured 1 wk before the supplement initiation were used as covariates for body temperature, DMI, milk yield, and milk composition. The covariance structure was autoregressive [AR(1)]. Data are presented as covariate-adjusted least squares means. Digestibility and plasma GABA data were analyzed by PROC GLM of SAS (SAS Institute, 2005). Treatment effects were tested using linear, quadratic, and cubic orthogonal polynomial contrasts. Standard errors of the mean are reported. Significance was declared at $P < 0.05$ and trends were discussed at $P < 0.10$.

RESULTS

Evaluation of Ruminally Protected GABA Product

The average rumen effective degradability, soluble fraction, potentially degradable fraction, and rate of degradation of ruminally protected GABA DM was 25.2, 0.8, 89.7% and 2.2%/h, respectively. The concentration of GABA in plasma increased linearly ($P = 0.06$) with the increasing levels of GABA (Table 1).

Environmental Conditions, Rectal Temperature, and Respiration Rate

The mean THI was 78.4 (range from 75.0 to 80.6) at 0700 h, 80.2 (range from 75.6 to 83.3) at 1400 h, and 78.7 (range from 74.8 to 80.8) at 2200 h (Figure 1). Rectal temperatures decreased linearly ($P < 0.05$) at 0700, 1400, and 2200 h with increasing GABA concentration (Table 1). Supplementation of GABA had no effect ($P > 0.05$) on respiration rates at any time point.

DMI, Milk Yield, Milk Composition, SCS, and Feed Efficiency

The effects of GABA treatment on DMI, milk yield, milk composition, SCS, and feed efficiency are shown in Table 2. The DMI, ECM, 4% FCM, and milk fat yield tended to increase linearly ($P < 0.10$) with increasing GABA. Supplementation of GABA affected, in a quadratic manner, milk protein and lactose concentrations ($P < 0.01$) and milk protein yield ($P = 0.06$), and the peak values were reached at a dose of 40 mg of GABA/kg. Content of MUN responded quadratically ($P < 0.05$) with increasing GABA concentration. Total solids content increased linearly ($P < 0.05$) with increasing GABA concentration. Supplementation of GABA had no effect on milk yield, lactose production, TS yield, milk fat concentration, SCS, or feed efficiency.

Figure 1. Temporal temperature-humidity index (THI) pattern at 0700, 1400, and 2200 h and mean during the 10-wk experimental period. Thresholds for no stress, mild stress, and high stress are from Armstrong (1994).

Diet Nutrient Digestibility

Apparent total-tract digestibilities of DM, OM, CP, NDF, and ADF were not affected ($P > 0.05$) by GABA supplementation (Table 3).

DISCUSSION

In the current study, the GABA product was rumen protected according to rumen effective degradability, soluble fraction, potentially degradable fraction, and rate of degradation of ruminally protected GABA. Moreover, the concentration of GABA in blood increased linearly with increasing GABA concentration, suggesting that the supplemented GABA was well absorbed into the blood.

Cows in the current study were exposed to heat stress, as the average THI in all pens reached values above 72 (Armstrong, 1994), but rectal temperature linearly decreased with increasing GABA, suggesting that feeding rumen-protected GABA could alleviate heat stress in dairy cows. This is in agreement with findings in rabbits (Frosini et al., 2003, 2006) and rats (Yakimova et al., 1996; Pierau et al., 1997). Moreover, Miyazawa et al. (2012) reported that oral administration of GABA induced a decrease in body core temperature compared with control conditions during rest in a hot environment. The GABA-transaminase inhibitor vigabatrin has been reported to induce hypothermia (Ginefri-

Gayet and Gayet, 1993) by raising brain GABA concentrations (Errante et al., 2002). Moreover, Ricci et al. (2008) reported neuroprotection provided by GABA against oxygen-glucose deprivation-induced injury in rat cortical brain slices. Therefore, the hypothermia in the present study may be due to the neuroprotection of GABA.

γ -Aminobutyric acid and its agonists appear to regulate feed intake in some mammalian animals through effects on both the central and peripheral nervous system. Injecting the GABA receptor agonist into the ventral tegmental area and the paraventricular nucleus increased animal feed intake (Echo et al., 2002; Stratford and Wirtshafter, 2004). Moreover, Ebenezer (1996) reported that systematic injection of baclofen, a GABA agonist, decreased the inhibitory effect caused by cholecystokinin on DMI in rats. In the current study, DMI tended to increase linearly with the increasing levels of GABA, which agree with previous results of Wang et al. (2013), who observed that DMI of dairy cows increased with rumen-protected GABA supplementation at levels of 0.8, 1.6, or 2.4 g/d, the actual predicted available amounts being 0.30, 0.61, or 0.91 g of GABA/d, respectively. The reasons for the higher DMI with increasing GABA concentration might be that GABA stimulates gastrin and digestive enzyme release, inhibits cholecystokinin release, weakens the negative feedback action of alimentary canal chyme on feed intake, and inhibits the gastric distension and satiety center, thus increasing

Table 2. Effects of rumen-protected γ -aminobutyric acid (GABA) on production variables in heat-stressed dairy cows

Item	Treatment ¹				SEM	P-value		
	Control	40GABA	80GABA	120GABA		Linear	Quadratic	Cubic
DMI (kg/d)	21.2	22.7	22.1	21.9	0.33	0.10	0.19	0.31
Production (kg/d)								
Milk yield	31.3	33.3	31.9	31.4	0.68	0.38	0.42	0.23
ECM ²	29.5	32.6	31.6	31.0	0.79	0.09	0.23	0.53
4% FCM ³	27.5	30.3	29.7	28.9	0.48	0.08	0.32	0.64
Fat yield	1.01	1.14	1.12	1.08	0.034	0.08	0.31	0.77
Protein yield	0.89	0.98	0.91	0.90	0.024	0.36	0.06	0.13
Lactose	1.54	1.65	1.57	1.57	0.032	0.49	0.27	0.35
TS	3.8	4.2	4.1	4.0	0.12	0.12	0.17	0.62
Milk component (%)								
Fat	3.23	3.44	3.48	3.45	0.094	0.15	0.38	0.41
Protein	2.84	2.97	2.86	2.91	0.037	0.40	<0.01	0.15
Lactose	4.89	4.96	4.93	4.99	0.013	0.72	<0.01	0.28
TS	12.21	12.74	12.80	12.76	0.124	0.01	0.10	0.35
MUN (mg/100 mL)	12.3	13.4	13.6	13.9	0.31	<0.01	0.02	0.03
SCS ⁴	3.22	3.94	3.40	3.61	0.471	0.67	0.33	0.70
Feed efficiency ⁵	1.48	1.46	1.44	1.43	0.021	0.12	0.56	0.48

¹Cows were fed a basal diet (control) or basal diet supplemented with 40, 80, or 120 mg of GABA/kg of DM.

²ECM = $0.327 \times \text{milk (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{protein (kg)}$.

³4% FCM = $0.4 (\text{kg of milk}) + 15.0 (\text{kg of fat})$.

⁴SCS = $\log_2 [\text{SCC (cells per mL)} / 100,000] + 3$.

⁵Feed efficiency = milk yield/DMI.

feed intake (Kato et al., 2001; Piqueras and Martinez, 2004). γ -Aminobutyric acid is an enhancer of neuropeptide Y (NPY), and is coexpressed with NPY in the central nervous system (Pu et al., 1999); thus, increased feed intake in cows fed GABA is also possibly associated with increased serum contents of GABA and NPY content in dairy cows (Wang et al., 2013). Furthermore, as heat stress can decrease feed intake (Huber, 1996), the tendency to increase DMI in cows fed GABA in this study may also be due to the alleviation of heat stress by GABA. However, several studies reported a nonsignificant influence in feed intake for steers infused with ruminally unprotected GABA into the rumen at levels up to 6 g/kg of DM (Dawson and Mayne, 1996) or 24 g/kg of DM (Cole, 1992). Furthermore, Buchanan-Smith (1982) observed that intraruminal infusion of 40 g of GABA/kg of DM reduced feed intake in sheep fed alfalfa pellets as the basal diet. Differences in feed

intake may be attributed to the differences in breed, GABA supplemental level, environment temperature, and the experimental diet.

The present results show an increase in ECM and FCM yield by supplementation of GABA, which agrees with the previous reported results of Wang et al. (2013), who observed that milk yield was increased in cows fed 0.8 g of GABA/d, whereas no further increment was observed when feeding 1.6 or 2.4 g of GABA/d. In agreement with the present study, GABA supplementation to chicks at 50 to 100 mg/kg alleviated the negative effects of heat stress, resulting in greater performance (Dai et al., 2011; Zhang et al., 2012; Xie et al., 2013). In pigs, GABA supplementation at 50 or 100 mg/kg also increased performance (Fan et al., 2007). In the present study, the increased DMI increased the net energy supply, which was probably one factor that increased the milk production of dairy cows.

Table 3. Effects of rumen-protected γ -aminobutyric acid (GABA) on apparent total-tract digestibility of nutrients in heat-stressed dairy cows

Apparent digestibility (%)	Treatment ¹				SEM	P-value		
	Control	40GABA	80GABA	120GABA		Linear	Quadratic	Cubic
DM	69.2	68.3	68.2	67.7	0.38	0.51	0.34	0.39
OM	70.7	70.2	70.1	69.3	0.41	0.84	0.42	0.36
CP	66.6	65.9	66.4	64.8	0.51	0.91	0.28	0.50
NDF	61.0	59.1	61.4	61.3	0.55	0.83	0.36	0.17
ADF	36.2	35.3	35.2	35.6	0.86	0.66	0.90	0.99

¹Cows were fed a basal diet (control) or basal diet supplemented with 40, 80, or 120 mg of GABA/kg of DM.

It has been documented that heat stress decreases milk protein concentration (Rhoads et al., 2009; Shwartz et al., 2009), but does not reduce milk fat content (Shwartz et al., 2009). In this study, milk fat content was not affected by GABA supplementation, which agrees with previous reported results (Wang et al., 2013). Morrison (1983) reported that the effect of experimental heat stress on milk fat is thought to be dependent on the interaction between dietary forage:concentrate ratios and temperature; thus, the reasons for no difference of the milk fat content in the current study may be that the dietary forage:concentrate ratios were the same among groups. The linear increase in milk fat yield with increasing GABA concentration in the present study may be due to the increased DMI. However, a quadratic relationship existed between the GABA doses and milk protein concentration, peaking at a dose of 40 mg of GABA/kg, which differs from Wang et al. (2013), who observed that milk protein content was not affected by GABA supplementation. Bernabucci et al. (2002) reported that heat itself (rather than reduced amino acid intake) affects milk protein-synthesizing machinery, especially α - and β -CN synthesis. Therefore, the increase in milk protein with 40 mg/kg GABA supplementation might be that GABA could affect milk protein synthesis, increase amino acid supply, or alleviate heat stress in dairy cows. This hypothesis warrants further research.

Several experiments indicated that heat stress decreased lactose concentration (Nardone et al., 1997; Shwartz et al., 2009). In the current study, the increase in milk lactose content with 40 mg/kg GABA supplementation may be due to the alleviation of heat stress. However, the result of the increasing MUN content with increasing GABA concentration found in this study is not consistent with previous results of Wang et al. (2013), who observed that MUN content was not affected by GABA supplementation. It is currently unclear whether GABA affects milk component synthesis directly or indirectly by influencing the delivery of component precursors.

CONCLUSIONS

Feeding GABA to dairy cows during the warm summer months alleviated heat stress by reducing rectal temperatures, increased DMI and milk production, and enhanced milk protein and lactose concentrations, but did not influence nutrient digestibility. In the present study, the optimal level of GABA supplementation for heat-stressed dairy cows was 40 mg/kg of DM.

ACKNOWLEDGMENTS

This study was supported financially by National Key Basic Research Program of China (Beijing, China; 2011CB100805), the Research Program of the State Key Laboratory of Animal Nutrition (Beijing, China; 2004DA125184F1102), National Key Technologies R & D Program (Beijing, China; 2012BAD12B02; 2012BAD12B08-5), and China Postdoctoral Science Foundation Funded Project (Beijing, China; 2012M520481).

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