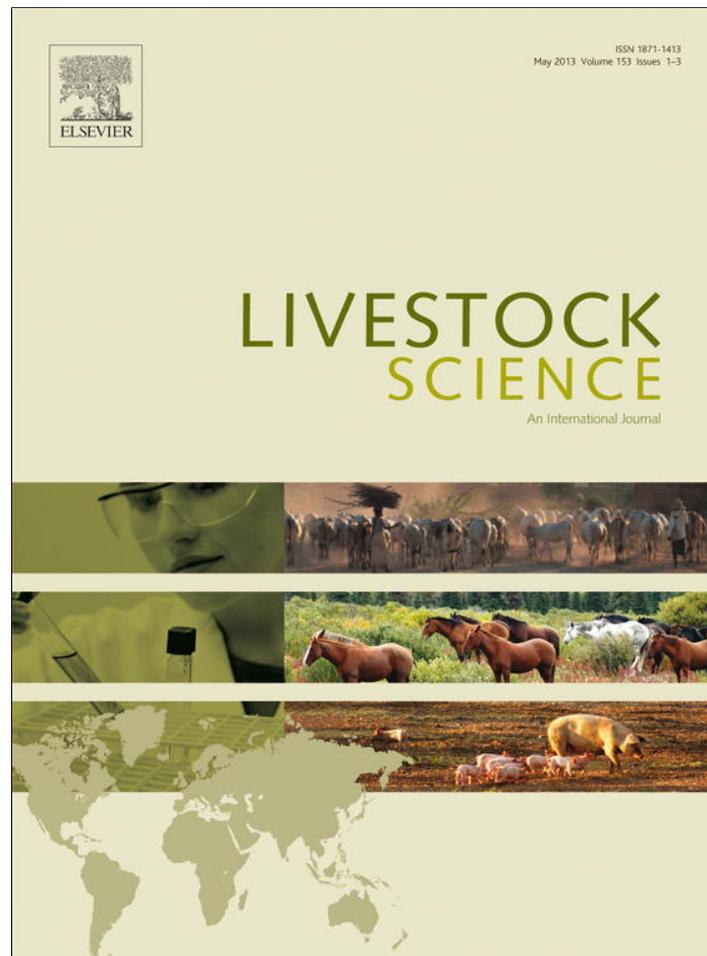


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.

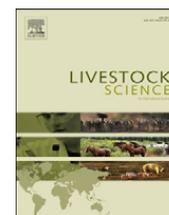


This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/authorsrights>



Effects of rumen-protected gamma-aminobutyric acid on feed intake, performance and antioxidative status in transition cows



D.M. Wang^a, H.Y. Liu^a, C. Wang^{a,b}, J.X. Liu^{a,*}, J.D. Ferguson^c

^a Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou 310029, PR China

^b Zhejiang Agriculture and Forestry University, Lin'an, Hangzhou 311300, PR China

^c Center for Animal Health and Productivity, University of Pennsylvania, School of Veterinary Medicine, PA 19348, United States

ARTICLE INFO

Article history:

Received 30 July 2012

Received in revised form

11 January 2013

Accepted 22 January 2013

Keywords:

γ-aminobutyric acid

Dry matter intake

Transition cows

ABSTRACT

The objective of this study was to investigate the effects of non-protected and rumen-protected gamma-aminobutyric acid (GABA) supplementation on dry matter intake (DMI), energy balance, milk performance, and serum metabolites in transition cows. Forty cows were blocked based on previous milk production, parity, estimated calving date and body weight and were randomly assigned to one of four treatments and received either no GABA (control) or were supplemented with non-protected GABA (0.6 g/day) or rumen-protected GABA (0.6 and 1.2 g/day), respectively. The experiment lasted from week 2 before calving to week 4 after calving. Milk yield and milk composition were recorded weekly. Serum concentrations of GABA, neuropeptide Y, cholecystokinin, leptin and biochemical and antioxidative metabolites were analyzed weekly. In week 3 and 4 after calving, DMI was higher ($P < 0.05$) in the cows fed 1.2 g/day rumen-protected GABA, compared with that of the control. In week 4 after calving, milk protein yield was higher in the cows fed 1.2 g/day rumen-protected GABA than that of the control cows. No differences were observed in the serum concentrations of GABA, neuropeptide Y and leptin among all the treatments, while non-esterified fatty acids and cholecystokinin concentrations decreased ($P < 0.01$) in cows fed rumen-protected GABA on day 15 and 22 after calving, respectively. Serum total antioxidative capacity and concentrations of glutathione peroxidase and superoxide dismutase were higher ($P < 0.05$) at several sampling points in cows fed rumen-protected GABA. In conclusion, supplementation of rumen-protected GABA at 1.2 g/day increased feed intake, improved milk protein yield, and decreased non-esterified fatty acids suggesting supplementation may be beneficial to postpartum dairy cows.

© 2013 Elsevier B.V. All rights reserved.

tr ct

The transition period is a critical and stressful time in the production cycle of a dairy cow (Goff and Horst, 1997). Body homeostasis and health are greatly challenged at

parturition, due to decreased feed intake and increasing milk production, resulting in significant endocrine and metabolic changes (Guo et al., 2007). It is reported that metabolic signals (leptin, neuropeptide Y (NPY) and non-esterified fatty acids (NEFA)) influence feed intake of transition cows (Ingvarstsen and Boisclair, 2001). Moreover, increased concentrations of cholecystokinin (CCK) might lead to decreased feed intake in dairy cattle (Choi and Dohald, 1996). Cows with transition problems may experience negative effects on production performance

* Corresponding author. Tel.: +86 571 88982097;

fax: +86 571 88982930.

E-mail address: liujx@zju.edu.cn (J.X. Liu).

and health (Overton and Waldron, 2004). Also, oxidative stress has negative effects on transition dairy cows (Castillo et al., 2005). A possible relationship between metabolic status and oxidative stress may exist in the transition period (Bernabucci et al., 2005). Evaluation of body oxidative status may be a complementary way to evaluate metabolic status (Castillo et al., 2003).

Gamma-aminobutyric acid (GABA) is a neurotransmitter, widely distributed in the central nervous system (CNS), peripheral nervous tissue, and non-neural tissue (Martin and Rimvall, 1993). GABA and its agonists appear to regulate feed intake in some mammalian animals through effects on both central and peripheral nervous systems. Kelly et al. (1979) reported that injecting muscimol (GABA-A receptor agonist) into the central hypothalamus increased food intake, while food intake decreased when muscimol was injected into lateral hypothalamus, suggesting that food intake regulatory effects of the GABA might not be similar in different organs. Higgs and Barber (2004) reported that low doses of baclofen, a GABA-B receptor agonist, could increase food intake by attenuating naturally occurring satiety signals. Moreover, GABA is an enhancer of NPY, and is co-expressed with NPY in the CNS (Pu et al., 1999; Shuye et al., 1999; Coppola et al., 2005). In the peripheral nervous system, GABA might regulate feed intake behavior through regulation of secretion of CCK (Ebenezer, 1996), which is synthesized in type I digestive tract cells (Liddle et al., 1986). Ebenezer and Warren (1993) reported that benzodiazepine diazepam, a GABA-A receptor agonist, attenuated the inhibitory effect of CCK on feed intake in food-deprived rats. These results showed that the GABA-B receptor may be involved in the CCK-related regulatory mechanism.

Previously, we observed that dietary rumen-protected GABA increased grass hay intake, milk yield, and milk protein yield (Wang et al., 2010b). However, information is limited about the effects of GABA in the transition dairy cow. The objective of this study was to determine the effects of dietary GABA (unprotected and rumen-protected) on feed intake, milk production, serum concentrations of NEFA, glucose, triglycerides, NPY, leptin, urea nitrogen, superoxide dismutase, malondialdehyde, and oxidative status assessed by measuring glutathione peroxidase activity and total antioxidative capacity in transition dairy cattle.

t r s t s

2.1. Animal, diets and experimental design

Forty multiparous Holstein dairy cows (parity 2.8 ± 0.67 , BW 606 ± 57.1 kg, previous milk yield 6943 ± 321.3 kg) were used. Experimental period included 15 days prior to calving through 28 days after parturition. Cows were assigned into 10 blocks based on predicted calving date and randomly assigned within block to one of four dietary treatments. The dietary treatments were 0 (control), 0.6 g/day non-protected GABA, 0.6 g/day rumen-protected GABA (supplemented as 1.2 g/day of 0.6 g of GABA coated with 0.6 g of palm oil), or 1.2 g/day rumen-protected GABA (supplemented as 2.4 g/day of 1.2 g of GABA coated with

1.2 g of palm oil) fed as a top-dress on TMR at the beginning of each daily feeding, respectively. Non-protected GABA (GABA purity > 99%) and rumen-protected GABA were both commercial products (Hangzhou King Techina Co. Ltd., China). Rumen-protected GABA, containing 50% GABA within an equal amount of palm oil coating, was estimated to be 82.0% rumen-protected after in situ incubation for 24 h, and abomasal release (Rossi et al., 2003) was 92.4% after in vitro incubation for 12 h (Wang et al., 2010a). Cows were kept in a tie-stall barn, and were fed and milked three times daily at 06:30, 13:30 and 19:30. All the cows had free access to water.

2.2. Sampling, measurement, and analyses

Cows were fed a total mixed ration (TMR) for ad libitum intake allowing 10% ort. The TMR amounts fed and refused were recorded weekly on two consecutive days (day 4 and 5) to determine dry matter intake (DMI). Composition of basal diets for prepartum and postpartum periods are presented in Table 1. Diets and ort samples were collected and analyzed for dry matter (DM), total nitrogen (method 984.13, AOAC, 2000), acid detergent fiber (ADFom; Van Soest et al., 1991) and neutral detergent fiber (NDFom; method 973.18, AOAC, 2000).

Milk yield was measured on day 4 of every week using the Waikato Milking System Meters (Waikato Milking Systems NZ Ltd., Hamilton, New Zealand). Milk samples were collected from all the animals on three consecutive milkings on day 4 each week, and analyzed for content

Ingredients and composition of the basal diets (DM basis).

| | Prepartum | Postpartum |
|---|-----------|------------|
| Ingredients (% of DM) | | |
| Ground corn grain | 18.8 | 28.5 |
| Wheat bran | 7.0 | 7.2 |
| Soybean meal | 0.0 | 9.0 |
| Sesame meal | 2.0 | 0.0 |
| Cottonseed | 1.6 | 0.0 |
| Cottonseed meal | 3.0 | 0.0 |
| DDGS ^a | 0.0 | 4.6 |
| Alfalfa meal | 0.0 | 10.0 |
| Sugar beet pulp pellet | 0.0 | 3.7 |
| Corn silage | 19.0 | 21.0 |
| Grass Hay | 46.0 | 14.0 |
| Limestone | 0.5 | 0.5 |
| Premix ^b | 0.4 | 0.5 |
| Salt | 0.4 | 0.3 |
| Sodium bicarbonate | 0.4 | 0.0 |
| Dicalcium phosphate | 0.9 | 0.7 |
| Composition | | |
| CP (% DM) | 11.4 | 14.9 |
| ADF (% DM) | 26.1 | 20.7 |
| NDF (% DM) | 42.3 | 31.8 |
| Ether extract (% DM) | 3.4 | 3.7 |
| NE _L (MJ/kg DM) ^c | 5.22 | 6.11 |

^a Dried distillers grains with solubles.

^b Formulated to provide (per kg of premix) 1,000,000 IU of vitamin A; 200,000 IU of vitamin D; 1250 IU of vitamin E; 14,000 mg of Zn; 100 mg of Se; 180 mg of I; 3000 mg of Fe; 40 mg of Co; 3000 mg of Mn; and 3000 mg of Cu.

^c Calculated based on individual feedstuffs in China recommendations (Ministry of Agriculture MOA, 2004).

of fat, protein, lactose, and milk urea nitrogen (MUN) (Laporte and Paquin, 1999) and GABA (Naini et al., 1993).

Blood samples were collected from the coccygeal vein at 11:00 a.m. on day –14, –7, 0, 1, 8, 15 and 22 relative to calving, respectively, and then centrifuged at $3000 \times g$ for 10 min to collect serum. Serum samples were frozen at -20°C , then thawed and used for analysis of GABA (Naini et al., 1993), NPY (McShane et al., 1992), CCK (Choi and Dohald, 1996), leptin (Delavaud et al., 2000), triglycerides (Lammoglia et al., 1996), NEFA (McCutcheon and Bauman, 1986), glucose (McCutcheon and Bauman, 1986), blood urea nitrogen (BUN; Rahmatullah and Boyde (1980)), malondialdehyde (MDA; Zhang et al., 2006), glutathione peroxidase activity (GSH-Px; Zhang et al., 2006), superoxide dismutase (SOD; Zhang et al., 2006) and total antioxidative capacity (T-AOC; Tang et al., 2009).

All the cows were weighed on day 5 of week –2, 1 and 4 relative to calving date, with the method described by Roche et al. (2007).

2.3. Calculations and statistical analysis

Milk net energy (MJ/kg) was calculated as $4.184 \times \text{milk yield (kg)} \times (0.00929 \times \text{fat} + 0.00563 \times \text{protein} + 0.00395 \times \text{lactose})$ (g/kg), as described in NRC (2001). Intake of net energy for lactation (NE_L) was calculated by the sum of net energy value of each feed ingredient multiplied by DMI and weighted by the proportion of the diet (Lucy et al., 1992). Energy balance (EB, MJ/day) before calving was equal to $4.184 \times [(DMI \times \text{diet } \text{NE}_L) - [(0.08 \times \text{BW}^{0.75}) + ((2 \times 0.00159 \times \text{days pregnant} - 0.0353) \times (\text{calf BW}/45)/0.14) \times 0.64]]$; EB after calving (MJ/day) was equal to $4.184 \times [(DMI \times \text{diet } \text{NE}_L) - [(0.08 \times \text{BW}^{0.75}) + (\text{milk } \text{NE}_L \times \text{milk yield})]]$, as described in NRC (2001).

All the data were analyzed using the PROC MIXED of SAS software system (SAS Institute, 2000). A randomized block design with repeated measures was used, with week, treatment, and interaction of treatment \times week as fixed effects, and cow within treatment as a random effect. The statistical model was as follows:

$$Y_{ij} = \mu + T_i + W_j + TW_{ij} + E_{ij}$$

where, Y_{ij} = dependent variable, μ = overall mean, T_i = treatment effect, W_j = week effect, TW_{ij} = interaction of treatment and week, E_{ij} = error term. Results are reported as least squares means. Probability values of $P < 0.05$ were used to define statistical significance and the values $P < 0.10$ and $P > 0.05$ were defined as statistical trends.

Results

3.1. Feed intake, energy balance, and lactation performance

Effects of dietary supplementation of rumen-protected GABA on DMI and EB status are presented in Fig. 1 and Table 2, respectively. The DMI increased ($P < 0.05$) for cows fed 1.2 g/day rumen-protected GABA in week 3 and 4 after calving, compared with the control group, cows fed non-protected GABA, and cows fed 0.6 g/day rumen-protected GABA (Fig. 1, $P < 0.05$). For cows fed 1.2 g/day rumen-protected GABA, DMI and crude protein (CP) intake

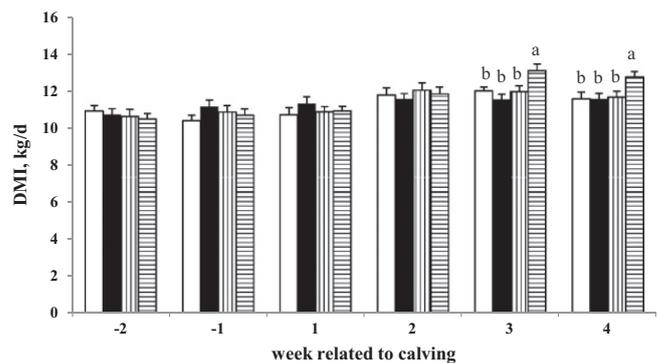


Fig. 1. Average dry matter intake (DMI) of transition dairy cows supplemented without gamma-aminobutyric acid (GABA) (□) or with 0.6 g/day non-protected GABA (■), 0.6 g/day rumen-protected GABA (▨) or 1.2 g/day rumen-protected GABA (▩) (n=10). ^{a,b} Means within the same week with different superscripts differ ($P < 0.05$).

increased ($P < 0.01$) in week 4 after calving, compared with the control group. The differences on neither DMI nor CP were not observed across all treatments in the week 2 before calving and week 1 after calving ($P > 0.10$), respectively. Feeding either rumen-protected or non-protected GABA did not alter EB status of the cows ($P > 0.05$, Table 2). However, EB decreased ($P < 0.01$) from week 2 before calving to week 1 after calving ($P < 0.01$), and from week 1 to 4 after calving, respectively. Negative EB status of cows fed 1.2 g/day rumen-protected GABA was alleviated compared to the control (-17.57 vs. -20.92 MJ/day) in week 4 after calving.

Milk yield during the experimental period was not different among the treatments ($P > 0.05$, Fig. 2(A) and Table 3). However, milk protein yield increased (Fig. 2(B), $P < 0.05$) for cows fed 1.2 g/day rumen-protected GABA in week 4 after calving, compared with the other groups. Milk composition (content of fat, protein and lactose), MUN, and GABA concentrations were not different among all treatments ($P > 0.05$; Table 3).

3.2. Serum variables

The results of serum variables are presented in Fig. 3. Serum CCK concentrations decreased in cows fed 1.2 g/day rumen-protected GABA on day 15 and 22 after calving ($P < 0.05$). Serum NEFA concentrations decreased in cows fed 0.6 or 1.2 g/day rumen-protected GABA on day 15 and 22 after calving compared to cows fed the control diet and 0.6 g/day rumen un-protected GABA ($P < 0.05$). Serum GSH-Px concentration was higher in cows fed rumen-protected GABA on day –14, 0, 15 and 22 relative to calving date than for cows fed the control diet and the diet with non-protected GABA ($P < 0.05$). Serum SOD concentration was higher in cows fed 1.2 g/day rumen-protected GABA on day –14, 15, and 22 relative to calving date, respectively, compared with the cows fed the control, non-protected GABA, and 0.6 g/day rumen-protected GABA ($P < 0.05$). On day of calving, serum SOD concentration was greater in cows fed 0.6 g/day rumen-protected GABA than for the control cows. Serum T-AOC concentration increased in cows fed both 0.6 and 1.2 g/day rumen-protected GABA

Effects of dietary supplementation of gamma-aminobutyric acid (GABA, non-protected; rumen-protected) on dry matter intake, crude protein intake and energy balance during –2nd week before calving and the 1st and 4th week after calving.

| t | r t ct | | r t ct | | |
|----------------------------|-------------------|-------------------|-------------------|-------------------|-------|
| | (/) | (/) | (/) | (/) | |
| s | 0 | 0.6 | 0.6 | 1.2 | |
| Dry matter intake (kg/day) | | | | | |
| 2nd week before calving | 10.9 | 10.7 | 10.6 | 10.5 | 0.33 |
| 1st week after calving | 10.7 | 11.3 | 10.9 | 10.9 | 0.34 |
| 4th week after calving | 11.6 ^a | 11.6 ^a | 11.7 ^a | 12.8 ^b | 0.32 |
| Crude protein (kg/day) | | | | | |
| 2nd week before calving | 1.24 | 1.22 | 1.22 | 1.20 | 0.038 |
| 1st week after calving | 1.59 | 1.68 | 1.62 | 1.62 | 0.051 |
| 4th week after calving | 1.72 ^a | 1.72 ^a | 1.74 ^a | 1.90 ^b | 0.048 |
| Energy balance (MJ/day) | | | | | |
| 2nd week before calving | 9.20 | 9.62 | 9.20 | 8.37 | 1.800 |
| 1st week after calving | –11.30 | –10.88 | –10.46 | –13.39 | 2.636 |
| 4th week after calving | –20.92 | –20.50 | –20.50 | –17.57 | 2.092 |

^a Means in the same row with different superscripts differ ($P < 0.05$).
^b Means in the same row with different superscripts differ ($P < 0.05$).

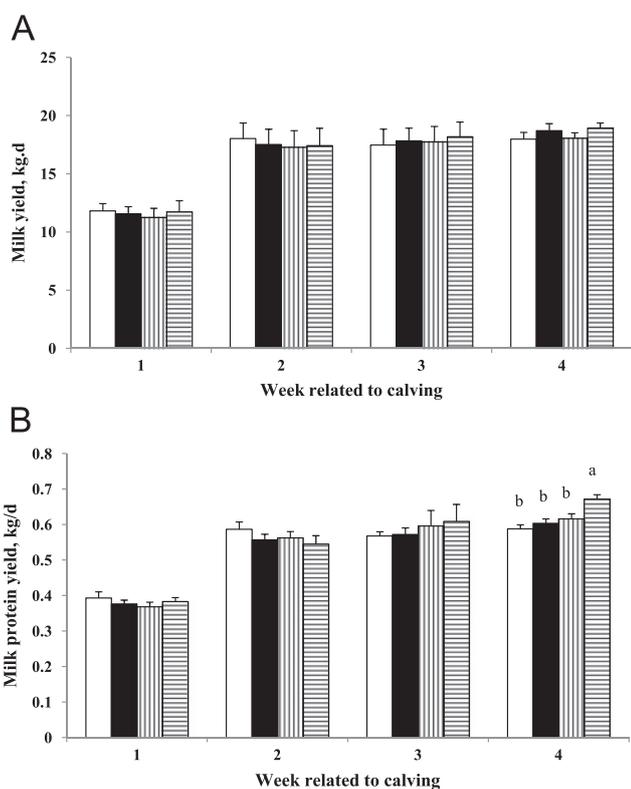


Figure A. Milk yield (kg/day,) and milk protein yield (kg/day,) of early lactation dairy cows supplemented without gamma-aminobutyric acid (GABA) (□) or with 0.6 g/day non-protected GABA (■), 0.6 g/day rumen-protected GABA (|||) or 1.2 g/day rumen-protected GABA (≡). Bars indicate standard error ($n=10$). ^{a,b} Means within the same week with different superscripts differ ($P < 0.05$).

on day 15 and 22 after calving, compared with the cows fed the control group and non-protected GABA, respectively ($P < 0.05$).

Serum concentrations of GABA, leptin, NPY, BUN and MDA were not different among all treatments ($P > 0.05$) throughout all sampling times (data not shown).

Effects of dietary supplementation of gamma-aminobutyric acid (GABA, non-protected; rumen-protected) on milk yield and milk compositions ^a in transition dairy cows.

| t | r t ct | | r t ct | | |
|-------------------------|--------|-------|--------|-------|-------|
| | (/) | (/) | (/) | (/) | |
| s | 0 | 0.6 | 0.6 | 1.2 | |
| Milk yield, kg/day | 16.3 | 16.4 | 16.1 | 16.6 | 0.55 |
| Milk composition | | | | | |
| Fat (%) | 3.69 | 3.63 | 3.78 | 3.82 | 0.068 |
| Protein (%) | 3.43 | 3.36 | 3.45 | 3.49 | 0.160 |
| Lactose (%) | 5.10 | 5.10 | 5.08 | 5.10 | 0.013 |
| MUN ^b (mg/L) | 117 | 111 | 109 | 11.0 | 3.6 |
| GABA (mmol/L) | 14.0 | 13.5 | 13.8 | 13.6 | 0.61 |

^a Milk compositions indicate the overall mean analyzed for milk samples collected from all the animals on three consecutive milkings weekly (4th day).
^b Milk urea nitrogen.

SC SS

In our previous study, supplementation of 0.4 g/day GABA increased the intake of grass hay, but did not alter serum concentration of GABA and NPY in peak lactating cows (Wang et al. 2010b). These results suggest that dietary GABA may regulate feed intake through pathways independent of NPY. It is well known that feed intake regulation includes both long-term and short-term effects. In short-term regulation, signals related to feed intake from the gastrointestinal (such as CCK) and other peripheral organs regulate current DMI through effects in the CNS (Richard, 2003). In long-term regulation, obesity signals (such as leptin) influence secretion from hypothalamic neurons to affect DMI (Richard, 2003). The GABA might regulate DMI by influencing CCK secretion from peripheral organs. In the study of Ebenezer (1996), systematic injection of baclofen, a GABA agonist, decreased the inhibitory effect caused by CCK on DMI in rats. In the present study, DMI of cows fed 1.2 g/day rumen-protected GABA

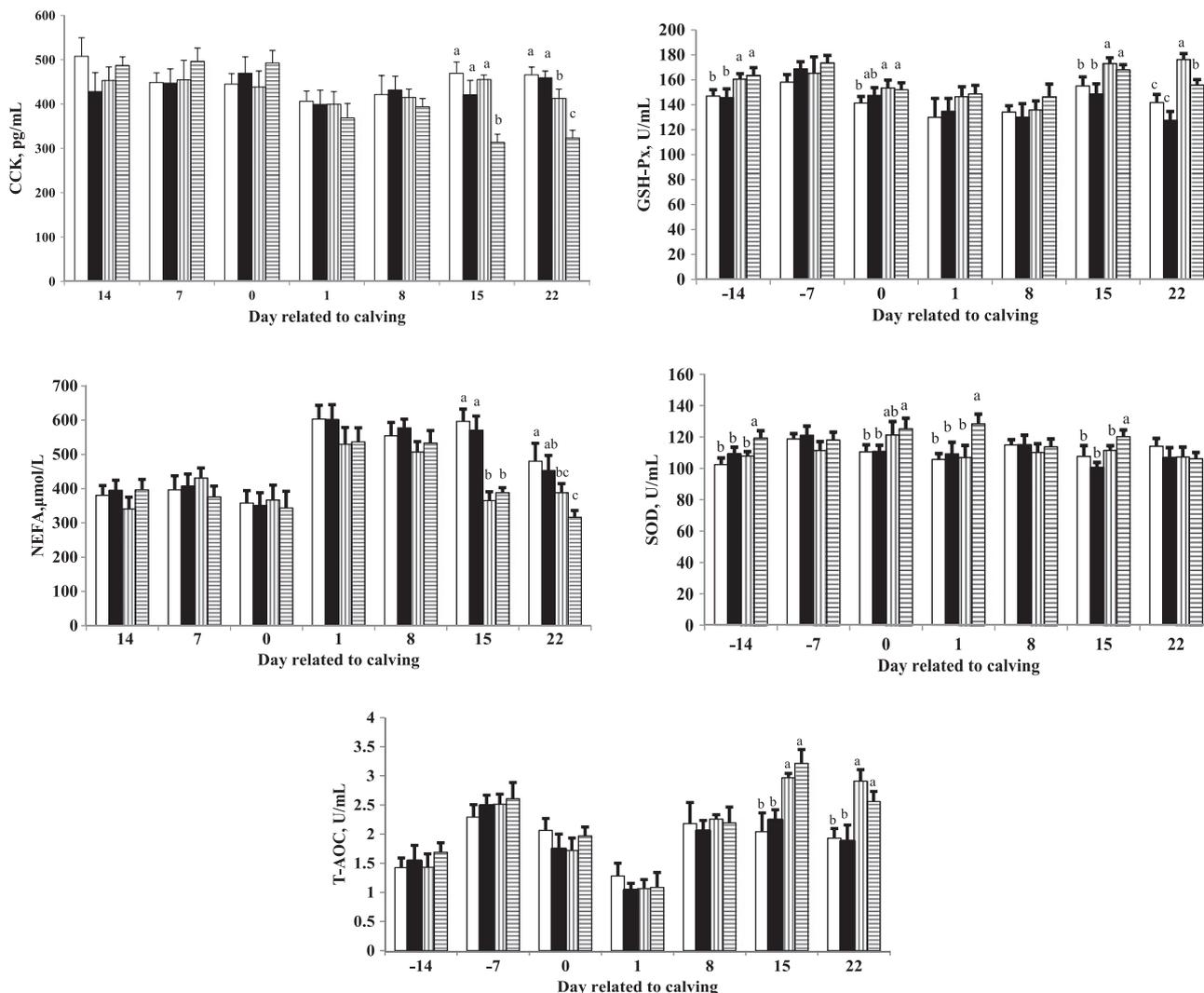


Fig. 3. Cholecystokinin (CCK), non-esterified fatty acids (NEFA) and antioxidative related variables in serum of transition dairy cows supplemented without gamma-aminobutyric acid (GABA) (□) or with 0.6 g/day non-protected GABA (■), 0.6 g/day rumen-protected GABA (▨) or 1.2 g/day rumen-protected GABA (▩). Bars indicate standard error (n=10). ^{a,b,c} Means within the same day with different superscripts differ (P < 0.05).

increased in week 3 and 4 after calving. Interestingly, serum CCK concentrations of animals fed rumen-protected GABA tended to decrease at this period (Fig. 3, P=0.10). The CCK is synthesized by type I cells of the gastrointestinal tract (mainly in the duodenum) (Liddle et al., 1986). These results suggest that dietary rumen-protected GABA may increase DMI in cows by inhibiting CCK synthesis or (and) secretion. Meanwhile, there was no difference in DMI between cows fed 0.6 g/day non-protected GABA and control cows (P > 0.05), suggesting that non-protected GABA is degraded by rumen microbes. No difference (P > 0.05) in serum GABA concentrations may be attributed to the low amount of the supplemented GABA (maximum at 1.2 g/day) and alternative use of absorbed GABA for other metabolic activities.

Increases in serum concentration of NEFA may indicate that feed intake was not sufficient to meet the demands of lactation (Ingvarstsen and Boisclair, 2001). Increased serum CCK concentration was associated with inhibited feed intake behavior in dairy cows (Choi and Dohald, 1996). From week -22 relative to calving, DMI was not

different (P > 0.05) among the four treatment groups. Serum CCK concentrations were not different (P > 0.05) among all the treatment groups in this period. This possibly accounted for the similar DMI and NEFA values across treatments in the first two weeks postpartum. Serum NEFA concentrations decreased in cows fed rumen-protected GABA in week 3 and 4 after calving (P < 0.05). During this period DMI was increased and serum CCK was decreased. The NEFA are considered a metabolic signal associated with negative EB (Ingvarstsen and Boisclair, 2001). For cows fed the control diet, DMI was lower than that in the cows fed 1.2 g/day rumen-protected GABA, suggesting that more body reserves were mobilized, resulting in an increase in serum NEFA concentrations. These results were consistent with our previous study (Wang et al., 2010b), that supplementation of rumen-protected GABA could effectively lowered serum NEFA concentrations of dairy cattle.

Wang et al. (2010c) showed a possible relationship between antioxidative status and EB. In the current study, serum concentrations of SOD, GSH-Px and T-AOC increased

in cows fed rumen-protected GABA, indicating that rumen-protected GABA reduced oxidative stress. Increased DMI in cows fed 1.2 g/day rumen-protected GABA improved the antioxidative status of transition dairy cows with alleviated energy balance status as well. Disorder of lipid metabolism was associated with decreased DMI in transition cows (Bell, 1995; Rukkwamsuk et al., 1999; Pysera and Opalka, 2000). A probable relationship between metabolic status and oxidative stress might exist in the transition period (Bernabucci et al., 2005). Thus, oxidative stress might be a serious problem in transition cows. Matsumoto et al. (2009) reported that supplemental GABA had a beneficial effect on group-housed calves using an automatic milk feeder. Supplementing GABA orally not only restrained stress but also improved secretion of IgA. In our previous study on early lactating cows (Wang et al., 2010b), rumen-protected GABA improved health condition of the cows. Results of the variables associated with antioxidative status indicate that dietary rumen-protected GABA might be beneficial for the antioxidative status of transition dairy cows.

Increased DMI in week 4 after calving of cows fed 1.2 g/day rumen-protected GABA enhanced CP intake and energy intake (Table 2). For cows fed 1.2 g/day rumen-protected GABA, concentrations of milk components in all postpartum period and milk yield of week 4 after calving was greater than that of the control cows, respectively ($P > 0.05$; Fig. 2(A) and Table 3). Thus, in the 4th week after calving, the increased milk protein yield in cows fed 1.2 g/day rumen-protected GABA was possibly due to the interaction of milk yield and milk content within this week. This result is similar with our previous study in early lactating cows (Wang et al., 2010b), in which 0.4 g/day GABA increased grass hay intake, milk yield and milk protein yield in lactating cows. However, in the present study, milk yield did not increase in cows fed rumen-protected GABA. The difference might be attributed to the different stages of lactation and different feeding strategies in the two studies. A long term study should be conducted to confirm this in the future.

c s s

Non-protected GABA had little effect on feed intake and lactation performance in transition cows. Supplementation of 1.2 g/day rumen-protected GABA could improve feed intake, milk protein yield, and health condition of the cows after calving. The results under this experiment indicated that supplementation of rumen-protected GABA at 1.2 g/day is beneficial for transition and especially for dairy cows after calving.

ct t r st st t t

None.

c t

This study was supported by the grants from the China Agriculture Research System (No.CARS-37) and Key Project of Science and Technology Department of Zhejiang

Province, China (No. 2008C12050). We acknowledge the staff of the Hangzhou Zhengxing Dairy Farm for their assistance with animal care.

r c s

AOAC, 2000. In: Official Methods of Analysis 17th ed. Association of Official Analytical Chemists, Arlington, VA.

Bell, A.W., 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73, 2804–2819.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.* 85, 2173–2179.

Castillo, C., Hernandez, J., Bravo, A., Lopez-Alonso, M., Pereira, V., Benedito, J.L., 2005. Oxidative status during late pregnancy and early lactation in dairy cows. *Vet. J.* 169, 286–292.

Castillo, C., Hernandez, J., Lopez-Alonso, M., Miranda, M., Benedito, J.L., 2003. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. *Arch. Anim. Breed* 46, 227–233.

Choi, B.R., Dohald, L.P., 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. *J. Nutr.* 126, 2913–2919.

Coppola, J.D., Horwitz, B.A., Hamilton, J., Blevins, J.E., McDonald, R.B., 2005. Reduced feeding response to muscimol and neuropeptide Y in senescent F344 rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, 1492–1498.

Delavaud, C., Bocquier, F., Chilliard, Y.D., Keisler, H., Gertler, A., Kann, G., 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *J. Endocrinol.* 165, 519–526.

Ebenezer, I.S., 1996. Baclofen pretreatment attenuates the suppressant effect of intraperitoneal administration of cholecystokinin (CCK) on food intake in rats. *Brain Res. Bull.* 41, 269–271.

Ebenezer, I.S., Warren, D., 1993. Diazepam pretreatment attenuates the suppressant effects of intraperitoneal administration of cholecystokinin (CCK) on food intake in food-deprived rats. *Methods Find. Exp. Clin. Pharmacol.* 15, 659–663.

Goff, J.P., Horst, R.L., 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* 80, 1260–1268.

Guo, J., Peters, R.R., Kohn, R.A., 2007. Effect of a transition diet on production performance and metabolism in periparturient dairy cows. *J. Dairy Sci.* 90, 5247–5258.

Higgs, S., Barber, D.J., 2004. Effects of baclofen on feeding behavior examined in the runway. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28, 405–408.

Ingvartsen, K.L., Boisclair, Y.R., 2001. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. *Domest. Anim. Endocrinol.* 21, 215–250.

Kelly, J., Rothstein, J., Grossman, S.P., 1979. GABA and hypothalamic feeding systems I. Topographic analysis of the effects of microinjections of muscimol. *Physiol. Behav.* 23, 1123–1134.

Lammoglia, M.A., Willard, S.T., Oldham, J.R., Randel, R.D., 1996. Effects of dietary fat and season on steroid hormonal profiles before parturition and on hormonal, cholesterol, triglycerides, follicular patterns, and postpartum reproduction in Brahman cows. *J. Anim. Sci.* 74, 2253–2262.

Laporte, M.F., Paquin, P., 1999. Near-infrared analysis of fat, protein, and casein in cow's milk. *J. Agric. Food. Chem.* 47, 2600–2605.

Liddle, R.A., Morita, E.T., Conrad, C.K., Williams, J.A., 1986. Regulation of gastric emptying in humans by cholecystokinin. *J. Clin. Invest.* 77, 992–996.

Lucy, M.C., Staples, C.R., Thatcher, W.W., Erickson, P.S., Cleale, R.M., Firkins, J.L., Clark, J.H., Murphy, M.R., Brodie, B.O., 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of postpartum ovulation and fertility in dairy cows. *Anim. Prod.* 54, 323–331.

Martin, D.L., Rimvall, K., 1993. Regulation of gamma-aminobutyric acid synthesis in the brain. *J. Neurochem.* 60, 395–407.

Matsumoto, D., Takagi, M., Fushimi, Y., Okamoto, K., Kido, M., Ryuno, M., Imura, Y., Matsunaga, M., Inokoshi, K., Shahada, F., Deguchi, E., 2009. Effects of gamma-aminobutyric acid administration on health and growth rate of group-housed Japanese Black Calves fed using an automatic controlled milk feeder. *J. Vet. Med. Sci.* 71, 651–656.

- McCutcheon, S.N., Bauman, D.E., 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. *J. Dairy Sci* 69, 44–51.
- McShane, T.M., May, T., Miner, J.L., Keisler, D.H., 1992. Central actions of neuropeptide-Y may provide a neuromodulatory link between nutrition and reproduction. *Biol. Reprod.* 46, 1151–1157.
- Ministry of Agriculture (MOA), PRC, 2004. Feeding Standard of Dairy Cattle (NY/T 34-2004). Beijing, China. (In Chinese).
- Naini, A.B., Vontzalidou, E., Cote, L.J., 1993. Isocratic HPLC assay with electrochemical detection of free gamma-aminobutyric acid in cerebrospinal fluid. *Clin. Chem.* 39, 247–250.
- NRC, 2001. Nutrient Requirements of Dairy Cattle, Natl. Acad. Sci. 7th rev. ed. National Research Council, Washington, DC.
- Overton, T.R., Waldron, M.R., 2004. Nutritional management of transition dairy cows: strategies to optimize metabolic health. *J. Dairy Sci.* 87, E105–E119.
- Pu, S., Jain, M.R., Horvath, T.L., Diano, S., Kalra, P.S., Kalra, S.P., 1999. Interactions between neuropeptide Y and gamma-aminobutyric acid in stimulation of feeding: a morphological and pharmacological analysis. *Endocrinology* 140, 933–940.
- Pysera, B., Opalka, A., 2000. The effect of gestation and lactation of dairy cows on lipid and lipoprotein patterns and composition in serum during winter and summer feeding. *J. Anim. Feed Sci.* 9, 411–424.
- Rahmatullah, M.T., Boyde, R.C., 1980. Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. *Clin. Chim. Acta* 107, 3–9.
- Richard, M.P., 2003. Genetic regulation of feed intake and energy balance in poultry. *Poult. Sci.* 82, 907–916.
- Roche, J.R., Macdonald, K.A., Burke, C.R., Lee, J.M., Berry, D.P., 2007. Associations among body condition score, body weight, and reproductive performance in seasonal-calving dairy cattle. *J. Dairy Sci.* 90, 376–391.
- Rossi, F., Maurizio, M., Francesco, M., Gionanna, C., Gianfranco, P., 2003. Rumen degradation and intestinal digestibility of rumen protected amino acids: comparison between in situ and in vitro data. *Anim. Feed Sci. Technol.* 108, 223–229.
- Rukkwamsuk, T., Kruip, A.M., Wensing, T., 1999. Relationship between overfeeding and overconditioning in the problems of high producing dairy cows during the postparturient period. *Vet. Q.* 21, 71–77.
- SAS Institute., 2000. SAS User's Guide: Statistics. Version 8.01. SAS Inst. Inc., Cary, NC.
- Shuye, P.U., Mukul, R.J., Tamas, L., Diano, S., Kalra, P.S., Kalra, S.P., 1999. Interactions between neuropeptide Y and gamma-aminobutyric acid in stimulation of feeding: a morphological and pharmacological analysis. *Endocrinology* 140, 933–940.
- Tang, Z., Yin, Y., Zhang, Y., Huang, R., Sun, Z., Li, T., Chu, W., Kong, X., Li, L., Geng, M., Tu, Q., 2009. Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin–lactoferrampin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21 d. *Br. J. Nutr.* 101, 998–1005.