Journal of Agricultural Science, Cambridge (1998), **131**, 321–327. *Printed in the United Kingdom*

Influence of duodenal infusion of betaine or choline on blood metabolites and duodenal electrical activity in Friesian calves

R. PUCHAŁA*, R. ZABIELSKI, V. LEŚNIEWSKA, M. GRALAK, P. KIELA AND W. BAREJ

Department of Animal Physiology, Warsaw Agricultural University, Nowoursynowska 166, 02 766 Warsaw, Poland

(*Revised MS received 20 April 1998*)

SUMMARY

Four, 4-week old Friesian calves (BW = 50 ± 3 kg), fitted with duodenal, portal and jugular catheters and duodenal electrodes, were used to study the metabolism of duodenally infused betaine (Bet) or choline (Chol) and their effects on blood sulphur amino acids and duodenal myoelectrical migrating complexes (MMC). Animals were fed milk replacer at 5% BW twice daily, but were starved overnight prior to the experimental procedure. Animals received a saline infusion for 2 h at 1 ml/min followed by a 1 h infusion of 1·2 or 3·6 mmol of either Bet or Chol. Infusion of saline was continued for another 2 h after the cessation of the amino acid infusion. Duodenal MMC were measured with a computer-based data acquisition system (MacLab, ADI, Australia). There were no differences in measured blood metabolites between the jugular and portal vein; therefore, only average values were presented. Plasma Met concentrations increased from 20 μM, 20 min after initiating Bet infusion, whereas a lower dose of Chol decreased plasma Met and a higher one had no effect. The highest plasma methionine (Met) concentration (29 μM) occurred 45 min after the onset of the Bet infusion (1·2 mmol). Compared to the 3·6 mmol Bet infusion, the intraduodenal infusion of 1·2 mmol of Bet resulted in a greater area ($P < 0·001$) under the plasma Met concentration curve (281·6 *v.* 73·3 mmol). A similar pattern was observed for plasma cystine concentrations. Infusion of Bet or Chol did not change the duration of MMC but Bet increased the number of spikes during the phase of low spiking activity (37·5 *v.* 14·6 pre-infusion, spikes/min; $P < 0·01$). Chol had the same effect but only after the infusion ceased (29·3 *v.* 11·5 spikes/min; $P < 0·01$). The velocity of migration of regular spiking activity (RSA; related to digesta transport) increased as a result of infusion (16·4 pre-infusion *v.* 31·3 Bet, 25·2 Chol cm/min; $P < 0·01$). Chol caused an immediate increase in the velocity of migration of RSA, whereas with the Bet infusion an increase was observed after cessation of infusion. Increased concentrations of sulphur amino acids during Bet infusion could indicate that labile methyl groups may be limited in calves. Postruminal Bet and Chol supplementation may cause a decrease in nutrient absorption in the small intestine by increasing digesta transport.

INTRODUCTION

Methionine (Met) serves two distinct functions in animal biochemistry: it both starts and participates in protein synthesis and it is a donor of methyl groups. With respect to the latter it is possible that compounds such as choline (Chol) or betaine (Bet) could partly replace Met (Fig. 1). Choline may be limiting for animal production, since an increase in milk fat percentage was observed by Erdman *et al.* (1984) as a result of dietary Chol supplementation in dairy cows. Betaine (Bet), an oxidative product of Chol, has received much less attention and was considered to be a nutritionally unimportant product of Chol catabolism. However, Storch *et al.* (1991) observed that 3 g/day oral Bet supplementation increased the Met concentration in plasma taken from human subjects. Bet appears to be an important metabolite in sulphur amino acid metabolism because betaine-homocysteine methyltransferase catalyses the methylation of homocysteine, using betaine as the methyl donor (Fig. 1). In an alternative pathway, 5-methyltetrahydrofolate

* Present address: Langston University, Langston, PO Box 730, OK 73050, USA. To whom all correspondence should be addressed. E-mail: rpuchala@luresext.edu

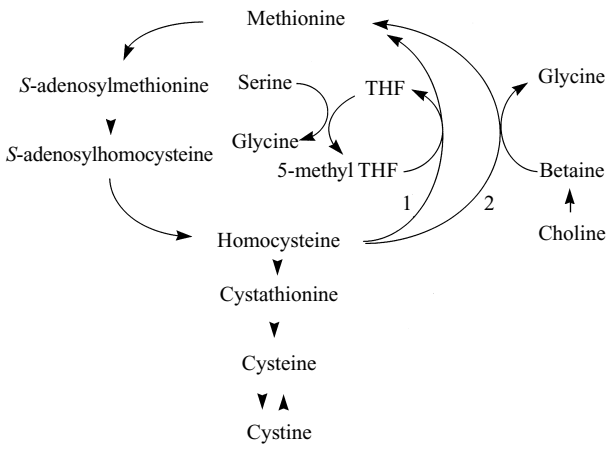

Fig. 1. Contribution of betaine and choline to methionine metabolism. Pathway 1 refers to 5-methyltetrahydrofolate homocysteine methyltransferase. Pathway 2 refers to betaine-homocysteine methyltransferase.

homocysteine methyltransferase regenerates Met using a methyl group derived from serine. Baker & Czarnecki (1985), using young chicks and rats, reported that Bet, but not Chol, showed efficacy in enhancing the conversion of high doses of homocysteine to Met. Therefore, it is important to know the effect of Bet or Chol delivered postruminally on sulphur amino acid metabolism.

Information on the absorption of nutrients from the intestine can be obtained from myoelectrical migrating complexes (MMC). These myoelectrical spikes are recorded from smooth muscle in the gastrointestinal tract and are considered to give useful information on the absorption of nutrients. Fioramonti *et al.* (1982) observed that increased glucose and xylose absorption in the jejunum was associated with more intense contractile activity during the phase of irregular spiking activity (ISA or phase II) of the MMC. Fioramonti & Bueno (1984) showed that mesenteric blood flow was positively related to intestinal motility. Kohn *et al.* (1993) demonstrated that the absorption of [^{14}C]L-lysine increased in parallel with increased blood flow to the intestine. Nutrients *per se* may modulate the motility pattern of the intestine, and thus affect their own absorption. For example, fat and fatty acids slow migration of regular spiking activity (RSA or phase III of MMC) in the upper part of the jejunum and increase their absorption and that of other nutrients (Wisen & Johansson 1992). A study by Puchała *et al.* (1997) showed that Met changed MMC and caused an increase in digesta flow in the small intestine.

The objective of this study was to determine the effects of duodenal infusions of Bet and Chol on intestinal absorption (by measuring MMC); and on the plasma concentrations of methionine and cystine.

MATERIALS AND METHODS

Animals

Four, 4-week old Friesian calves (BW = 50 ± 3 kg) were purchased from the commercial farm of Warsaw Agricultural University. They received milk replacer (at 5 % BW per feeding) twice daily at 08.00 and 20.00 h. The milk replacer (Pro Milk D; protein 22 %, fat 17 %, ash 10 %, LOL Agra International, Warsaw, Poland) was mixed with water (1 : 9). The calves were surgically fitted with silicone catheters implanted in the lumen, 1 cm posterior to the duodenal bulb, a silicone portal vein catheter (Silastic, Dow Corning Corp., Midland, Michigan, USA), and three bipolar silver electrodes, two in the duodenum and one in the upper jejunum (Zabielski *et al.* 1993). On recovery from anaesthesia (5–6 h), animals were housed in individual cages and allowed to recover for 1 week. The calves received daily intramuscular injections of procaine penicillin G (600000 IU; Polfa, Kutno, Poland) for 5 days post-operatively. Prior to treatment administration, animals were starved overnight and a catheter was inserted into the right external jugular vein under local anaesthesia 3 h before sampling. All calves had good appetites and appeared clinically healthy throughout the entire study.

Treatment and sampling

The animals received an intraduodenal saline infusion (1 ml/min) for 2 h followed by a 1 h infusion of 1·2 or 3·6 mmol of either Bet or Chol and finally a further 2 h infusion of saline (1 ml/min). Duodenal MMC were measured using an Apple computer-based data acquisition system (MacLab, ADI,

https://doi.org/10.1017/S0021859698005814 Published online by Cambridge University Press

Australia) as described by Zabielski *et al.* (1995). Only one animal was allocated to treatment each day and was fed at the conclusion of the experimental protocol. Blood samples were drawn from the jugular veins at −30, −15 and 0 min prior to metabolite infusions and thereafter every 5 min for 45 min and every 15 min for a further 2 h. Blood from the portal vein was drawn at similar intervals with the exception of the final 2 h when blood was drawn at 30 min intervals. Blood samples were collected on ice into 7 ml tubes containing sodium heparin (Becton Dickinson Vacutainer Systems, Rutherford, NJ) and centrifuged at 1500 ***g*** and 4 °C for 10 min. Plasma (0·45 ml) was immediately deproteinized for amino acid estimation using 0·05 ml of 50% 5-sulphosalicylic acid with internal standards added (sarcosine and norvaline). The mixture was vortexed and centrifuged (1500 ***g***; 4 °C; 10 min). The supernatant for amino acid analysis and remaining plasma was stored at −55 °C.

Analysis

Plasma amino acids were analysed as described by Puchała *et al.* (1994). Secondary amino acids were measured using the ion exchange method (lithium system) with postcolumn Trione ninhydrin derivatization (Pickering, Mountain View, CA). Plasma NEFA concentrations were analysed using a Wako NEFA test kit that utilizes an enzymatic method for the quantification of non-esterified (or free) fatty acids (Wako Pure Chemical Industries, Osaka, Japan). Plasma triglyceride concentrations were analysed colorimetrically using a Sigma diagnostic kit (Sigma Diagnostic, St. Louis, MO). Three-phased duodenal MMC were analysed for duration of particular phases (min), number of spikes/phase and the velocity of propagation of RSA phases along the duodenum and proximal jejunum (cm/min).

Statistical analysis

Analysis of variance was used to detect increases in plasma metabolite concentrations above a base-line and changes in electrical activity (SAS 1990). Means for each parameter were compared using a protected least-significant difference technique (Steel & Torrie 1980). The effect of each compound on plasma amino acid concentrations was tested using area-under-the-curve (AUC) analyses with AUC values compared by ANOVA and using orthogonal polynomials. Plasma amino acid data for each calf were curve-fitted by orthogonal polynomials to determine the cubic time effect score for each treatment. A randomized block design was used to test for differences among the cubic time-effect scores. The randomized block analysis is equivalent to performing a *t*-test on the curves where two treatments are given.

RESULTS

There were no differences in measured blood metabolites between the jugular and portal vein, therefore only average values are presented. Intraduodenal betaine infusion increased plasma Met, cystine (Cys) and glycine (Gly) concentrations (Fig. 2). The increase was higher for the lower dose of betaine (1·2 mmol). The mean 3 h AUC for the plasma Met concentration–time curve was 281·6 ± 34·36 μM/min for 1·2 mmol of Bet, whereas the AUC for 3·6 mmol of Bet was 73·3 ± 21·42 μM/min ($P < 0·001$). The AUC for the plasma Cys concentration–time curve was 428·7 ± 59·48 μM/min for 1·2 mmol of Bet and 131·6 ± 39·42 μM for 3·6 mmol of Bet ($P < 0·001$). AUC for Gly was also higher for the lower level of infusion (4226·7 ± 279·27 *v.* 1367·7 ± 182·31 for 3·6 mmol of Bet; $P < 0·01$). The increase in plasma Met was observed after 20 min of Bet infusion. Infusion of a lower dose of Chol (Fig. 3) decreased Met concentration (21·8 ± 1·24 μM pre-infusion *v.* 18·1 ± 1·33 during and post-infusion, $P < 0·05$) and caused a spike in plasma Cys concentration during infusion (42·4 ± 1·82 μM pre-infusion *v.* 50·2 ± 2·75 μM during infusion, $P < 0·05$). The higher dose of Chol had no effect on plasma Met and decreased Cys concentration during infusion. Plasma homocysteine was similar for Bet and Chol infusion, average pre-infusion concentration (8·5 ± 2·65 μM) was similar to that observed during infusion and post-infusion (8·1 ± 2·92 and 8·6 ± 2·41, respectively; $P > 0·48$). There were no changes in other analysed blood parameters due to intraduodenal infusions of Bet or Chol.

Infusion of Bet or Chol had no effect on the duration of MMC (Table 1). However a trend towards an increased duration of ISA was observed as a result of intraduodenal infusion of the higher dose of both metabolites. A decreased number of spikes during ISA (Table 2) was observed as a result of infusing the higher dose of both metabolites. Infusion of Bet caused an immediate increase in the number of spikes during low spiking activity (LSA) (Table 2; 37·5 *v.* 14·6 spikes/min; $P < 0·01$). Chol had the same effect but only after the infusion ceased (29·3 *v.* 11·5 spikes/min; $P < 0·01$). Infusion of Chol had an immediate effect on the velocity of the migration of RSA (Table 3; 25·2 *v.* 18·1 cm/min pre-infusion; $P < 0·01$). In contrast, the migration of the RSA increased only when the Bet infusion ceased (31·3 *v.* 16·4 cm/min pre-infusion; $P < 0·001$).

DISCUSSION

In this study, increases in Met, Cys and Gly were observed as a result of intraduodenal Bet infusions (Fig. 2). Infusion of the lower dose of Chol decreased Met concentration, whereas the higher dose of Chol had no effect on plasma Met. Storch *et al.* (1991)

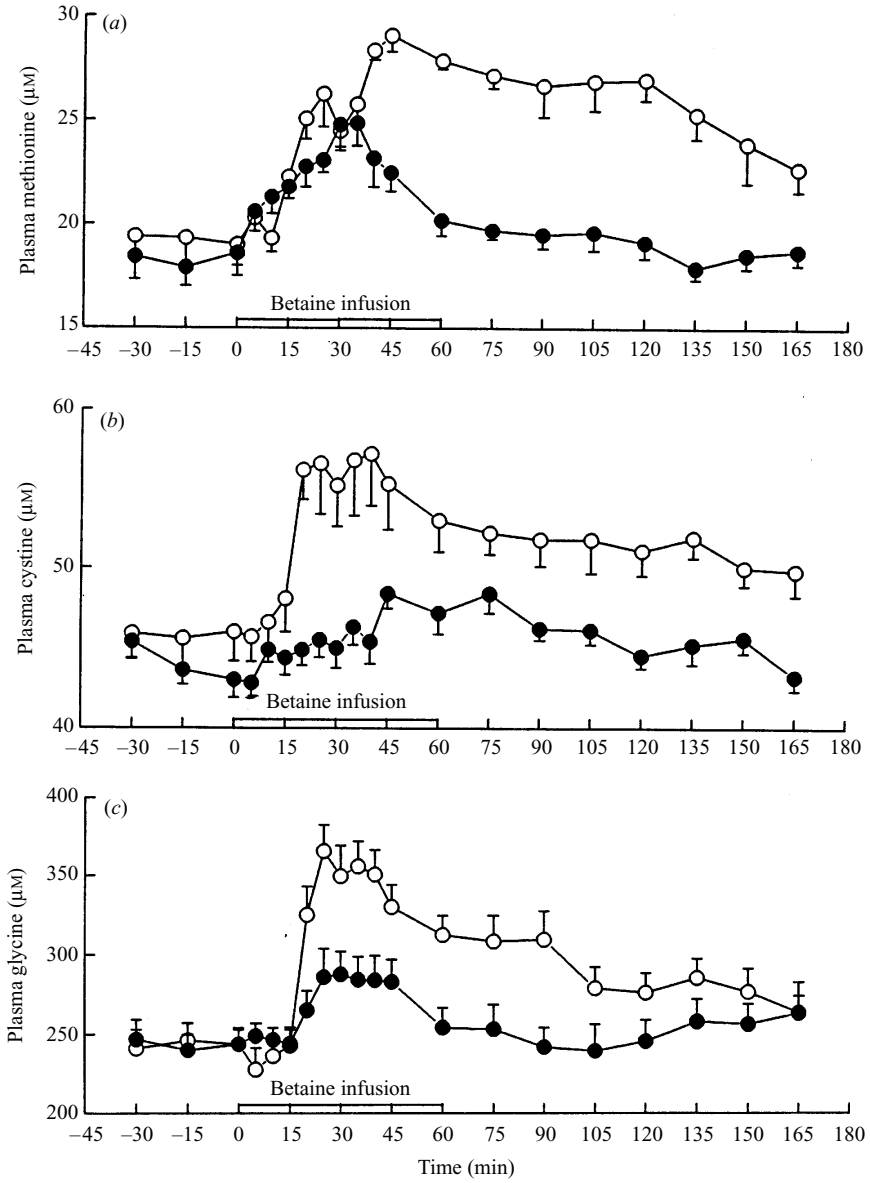

Fig. 2. Plasma (*a*) methionine, (*b*) cystine and (*c*) glycine concentrations (μM) during 1 h duodenal infusion of 1·2 mmol (○) or 3·6 mmol (●) of betaine. (D.F. = 7).

observed that Met concentration in plasma obtained from fed human subjects was significantly higher following 3 g d^{-1} oral Bet supplementation. Maree *et al.* (1990) found that many patients with vitamin B_{12} or folate deficiencies have normal serum concentrations of Met after Bet supplementation. This suggests a role of Bet-dependent Met synthesis in the maintenance of serum Met concentrations after Bet supplementation. The inability of Chol (decrease with lower dose of choline and lack of changes with higher dose) to increase plasma Met concentration as observed in this paper is consistent with the lack of effect on conversion of homocysteine to Met reported by Baker & Czarnecki (1985). It might be a result of a limited rate of conversion of Chol to Bet by choline dehydrogenase. This process requires an unusual type of dehydrogenase in that it is a flavoprotein that interacts directly with cytochrome *c* (Bender 1985). That there was an actual decrease in plasma Met and increase in plasma Cys with infusion of a low dose of

https://doi.org/10.1017/S0021859698005814 Published online by Cambridge University Press

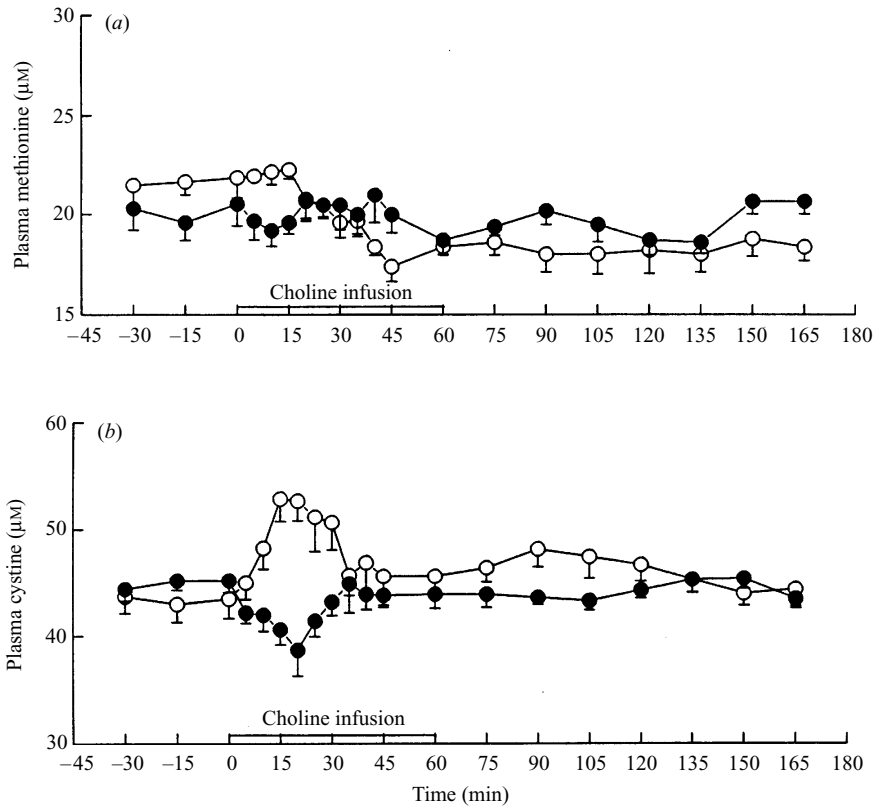

Fig. 3. Plasma (*a*) methionine and (*b*) cystine concentrations (μM) during 1 h duodenal infusion of 1·2 mmol (○) or 3·6 mmol (●) of choline. (D.F. = 7).

Table 1. *Effect of intraduodenal infusion of betaine or choline on the duration (min) of low spiking activity (LSA), irregular spiking activity (ISA) and regular spiking activity (RSA) phases of the duodenal motor migrating complexes (MMC) in calves (D.F. = 7)*

	LSA	ISA	RSA
		Choline	
Pre-infusion	10·2 ± 0·86	25·6 ± 2·18	2·7 ± 0·13
Infusion 1·2 mmol/h	10·9 ± 3·46	22·6 ± 3·03	2·6 ± 0·12
Post-infusion	11·0 ± 0·81	26·5 ± 4·11	2·2 ± 0·13
Infusion 3·6 mmol/h	11·1 ± 1·06	29·6 ± 2·58	2·6 ± 0·13
Post-infusion	10·8 ± 0·59	28·3 ± 1·82	2·6 ± 0·16
		Betaine	
Pre-infusion	9·5 ± 0·57	24·8 ± 2·29	2·8 ± 0·15
Infusion 1·2 mmol/h	10·5 ± 1·28	22·2 ± 3·34	2·5 ± 0·17
Post-infusion	9·2 ± 1·06	24·7 ± 3·51	2·8 ± 0·15
Infusion 3·6 mmol/h	10·8 ± 1·16	29·3 ± 1·58	2·7 ± 0·30
Post-infusion	11·1 ± 1·59	32·1 ± 2·56	2·5 ± 0·20

Table 2. *Effect of intraduodenal infusion of betaine or choline on the number of spikes (spikes/phase) in low spiking activity (LSA), irregular spiking activity (ISA) and regular spiking activity (RSA) phases of duodenal motor migrating complexes (MMC) in calves (D.F. = 7)*

	LSA	ISA	RSA
		Choline	
Pre-infusion	11·5 ± 1·8	2940 ± 550	1160 ± 90
Infusion 1·2 mmol/h	28·5 ± 18·8	2370 ± 370	1250 ± 370
Post-infusion	25·3 ± 6·3	2870 ± 170	1290 ± 120
Infusion 3·6 mmol/h	21·3 ± 7·5	1890 ± 590	950 ± 140
Post-infusion	29·3 ± 5·6	1779 ± 281	920 ± 110
		Betaine	
Pre-infusion	14·6 ± 2·3	2760 ± 570	1290 ± 100
Infusion 1·2 mmol/h	35·1 ± 7·1	2130 ± 420	1220 ± 160
Post-infusion	20·1 ± 4·4	2240 ± 350	1340 ± 110
Infusion 3·6 mmol/h	37·5 ± 2·9	1580 ± 250	1290 ± 260
Post-infusion	21·5 ± 5·9	2370 ± 560	1050 ± 170

https://doi.org/10.1017/S0021859698005814 Published online by Cambridge University Press

Table 3. *Effect of intraduodenal infusion of betaine or choline on the velocity of migration of regular spiking activity (RSA) in calves (cm/min; (D.F. = 7)*

	Migration of RSA	
	Choline	Betaine
Pre-infusion	17·1 ± 0·90	16·4 ± 1·20
Infusion	25·2 ± 1·20	16·2 ± 1·80
Post-infusion	19·0 ± 0·90	31·3 ± 1·30

Chol may indicate a stimulation of Met utilization – perhaps increased transsulphuration which, as Fig. 1 indicates, might result in increased Cys. The absence of changes in Met and the decrease in Cys with higher Chol seems to support the theory that Chol affects the transsulphuration process.

Barak & Tuma (1983) proposed that Bet, instead of merely being a metabolic by-product of Chol oxidation, may also serve as an important methylating agent when normal methylating pathways are impaired by ethanol ingestion, drugs or nutritional imbalances. Awad *et al.* (1983) presented evidence that Bet can replace *S*-adenosylmethionine as a source of a methyl group in processes such as methylation of nucleic acids and others that require methyl group transfer only from Met or *S*-adenosylmethionine. In this experiment, the increase in plasma Met concentration appeared to be partly due to both homocysteine remethylation and to the direct utilization of the methyl group supplied by Bet. The absence of changes in homocysteine concentrations as a result of Bet infusion may suggest that homocysteine remethylation is only partly responsible for increases in Met concentration. Direct utilization of methyl groups derived from Bet caused decreased requirement for *S*-adenosylmethionine (Met) that resulted in increased Met concentration. Bet is able to replace Met (*S*-adenosylmethionine) as a methyl donor and supports (or even replaces) Met in other physiologically important body processes. Such a replacement could contribute to higher concentrations of Met in the blood after supplementation with Bet (Awad *et al.* 1983).

Lower increases in plasma Met concentrations during introduodenal infusion of the higher dose (3·6 mmol) of Bet may be due to an overloading effect and/or a decreased absorption of Bet. It is possible that the high Bet concentration decreased its own and other metabolites' absorption from the intestine. A regulating effect of Met on metabolite transport was suggested by Hamaguchi *et al.* (1991) who observed that increased concentration of Met decreased cell transport of Met and other metabolites. A decreased number of spikes during ISA was observed during the infusion of the higher dose of Bet which supports the down-regulating effect of a high duodenal concentration of Bet on its absorption from the intestine. Bueno & Fioramonti (1994) documented that the greatest absorption of nutrients from the intestine occurred during ISA and was related to the intensity of the electric activity.

The increased plasma Gly concentrations observed as a result of Bet infusion (Fig. 2) could be due to demethylation of Bet if trimethyl glycine, the initial product, is readily converted to glycine. Increased Met concentrations at the same time suggested that methyl groups were used to regenerate Met from homocysteine.

Infusion of Bet or Chol affected the duration of MMC (Table 1). A tendency for increased duration of ISA was observed as a result of intraduodenal infusion of the higher doses of both metabolites. The increased duration of ISA was associated with a decreased number of spikes (Table 2). Both of these changes may be indicative of a decrease in absorption of nutrients from the intestine (Zabielski *et al.* 1993). Infused metabolites had different effects on the number of spikes during LSA. Infusion of Bet immediately increased the number of spikes during LSA but Chol had the same effect only after the cessation of Chol infusion. An increased number of spikes during LSA may be indicative of increased muscle activity and thereby improved digestion – perhaps through an increase in the mixing of digesta and pancreatic enzyme action. Infusion of either metabolite, however, resulted in an increased velocity of migration of RSA (Table 3). This may mean there was reduced time over which nutrients could be absorbed. The infusion of Chol had an immediate effect on the velocity of the migration of RSA whereas the infusion of Bet increased the migration of RSA after cessation of infusion. This may partly explain the differences in their action on metabolism of sulphur amino acids. The immediate effect of Chol on the migration of RSA probably impaired Chol absorption. In the case of Bet, absorption was not affected for the duration of infusion.

REFERENCES

AWAD, W. M., JR, WHITNEY, P. L., SKIBA, W. E., MANGUM, J. H. & WELLS, M. S. (1983). Evidence for direct methyl transfer in betaine: homocysteine *S*-methyltransferase. *Journal of Biological Chemistry* **258**, 12790–12792.

BAKER, D. H. & CZARNECKI, G. L. (1985). Transmethylation of homocysteine to methionine: efficiency in the rat and chick. *Journal of Nutrition* **115**, 1291–1299.

BARAK, A. J. & TUNA, D. J. (1983). Betaine, metabolic by-product or vital methylating agent? *Life Science* **32**, 771–774.

BENDER, D. A. (1985). *Amino Acid Metabolism*, *Second Edn*. Chichester: John Wiley & Sons.

BUENO, L. & FIORAMONTI, J. (1994). Neurohormonal control of intestinal transit. *Reproduction, Nutrition and Development* **34**, 513–525.

ERDMAN, R. A., SHAVER, R. D. & VANDERSALL, J. H. (1984). Dietary choline for the lactating cow: possible effects on milk fat synthesis. *Journal of Dairy Science* **67**, 410–415.

FIORAMONTI, J. & BUENO, L. (1984). Relation between intestinal motility and mesenteric blood flow in the conscious dog. *American Journal of Physiology* **246**, G108–G113.

FIORAMONTI, J., BUENO, L. & RUCKEBUSCH, M. (1982). Blood sugar oscillations and duodenal migrating myoelectric complexes. *American Journal of Physiology* **242**, G15–G20.

HAMAGUCHI, T., AZUMA, J. & SCHAFFER, S. (1991). Interaction of taurine with methionine: inhibition of myocardial phospholipid methyltransferase. *Journal of Cardiovascular Pharmacology* **18**, 224–230.

KOHN, R. A., SCHWAB, C. G., BOZAK, C. K. & HYLTON, W. E. (1993). Effect of intestinal blood flow on absorptive site blood flow and lysine absorption as examined in situ in Holstein calves. *Journal of Animal Science* **71**, 1641–1647.

MAREE, K. A., VAN DER WESTHUYZEN, J. & METZ, J. (1990). Interrelationship between serum concentrations of methionine, vitamin B12 and folate. *International Journal of Vitaminology and Nutritional Research* **60**, 136–141.

PUCHAŁA, R., PIÓR, H., VON KEYSERLINGK, M., SHELFORD, J. A. & BAREJ, W. (1994). Determination of methionine sulfoxide in biological materials using HPLC and its degradability in the rumen of cattle. *Animal Feed Science and Technology* **48**, 121–130.

PUCHAŁA, R., ZABIELSKI, R., LEŚNIEWSKA, V., KIELA, P. & BAREJ, W. (1997). Influence of duodenal infusion of L-methionine and L-methionine-dl-sulfoxide on amino acid concentration and duodenal electrical activity in Friesian calves. *Animal Feed Science and Technology* **66**, 75–84.

STATISTICAL ANALYSIS SYSTEM (1990) *SAS/STAT User's Guide*, *Version 6*, *Fourth Edn*. Cary, NC: SAS Institute.

STEEL, R. G. D. & TORRIE, J. A. (1980). *Principles and Procedures of Statistics*. New York: McGraw-Hill Book Co.

STORCH, K. J., WAGNER, D. A. & YOUNG, V. R. (1991). Methionine kinetics in adult men: effects of dietary betaine on L-[$^{2}H_{3}$-methyl-1-^{13}C]methionine. *American Journal of Clinical Nutrition* **54**, 386–394.

WISEN, O. & JOHANSSON, K. (1992). Gastrointestinal function in obesity: motility, secretion, and absorption following a liquid test meal. *Metabolism* **41**, 390–395.

ZABIELSKI, R., ONAGA, T., MINEO, H. & KATO, S. (1993). Periodic fluctuations in pancreatic secretion and duodenal motility investigated in neonatal calves. *Experimental Physiology* **78**, 675–684.

ZABIELSKI, R., ONAGA, T., MINEO, H., KATO, S. & PIERZYNOWSKI, S. G. (1995). Intraduodenal cholecystokinin octapeptide (CCK-8) can stimulate pancreatic secretion in the calf. *International Journal of Pancreatology* **17**, 271–278.