Periodic fluctuations of gut regulatory peptides in phase with the duodenal migrating myoelectric complex in preruminant calves: effect of different sources of dietary protein

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Four preruminant calves with implanted electrodes in the duodenum and a catheter in the external jugular vein were used for investigation of plasma gut regulatory peptide profiles during different phases of migrating myoelectric complex (MMC) in the small intestine. The effects of different dietary proteins on the rhythmic activity of gut peptides and gastrointestinal motility were compared. In particular, the effects of skimmed-milk protein (retaining physiological patterns of abomasal clotting, and abomaso-intestinal digesta flow) *v*. fish protein (devoid of clotting activity and modifying the digesta flow) were studied. In calves fed on the milk diet, plasma concentrations of pancreatic polypeptide, motilin, secretin, cholecystokinin (CCK) and somatostatin, but not vasoactive intestinal polypeptide or gastrin, fluctuated in phase with the duodenal MMC in the preprandial period. Feeding transiently affected the intestinal MMC and abolished the peptide fluctuations in a specimen-specific manner. In contrast, calves fed on the fish-protein diet showed more profound changes in intestinal MMC. In these animals the MMC-related fluctuations were significant only for plasma CCK. In conclusion, the source of dietary protein has an impact on the physiological endocrine function of the small intestine. Observed fluctuations of plasma gut regulatory peptides seem to be secondary to duodenal motility cycles.

Gut peptides: Duodenum: Myoelectrical activity: Dietary protein

The gastrointestinal (GI) tract of single-stomached animals manifests rhythmic activities of 1–2 h duration. These activities mainly concern GI motility and secretion, including the endocrine secretion (DiMagno *et al.* 1979; Vantrappen *et al.* 1979; Keane *et al.* 1980; Magee & Naruse, 1983). In dogs and human subjects, plasma pancreatic polypeptide (PP) and motilin concentrations fluctuate in phase with the duodenal migrating myoelectric complex (MMC) (Schwartz *et al.* 1979; Keane *et al.* 1980; Chen *et al.* 1983; Lee *et al.* 1986; Qvist *et al.* 1990). In contrast, the concentrations of plasma secretin and cholecystokinin (CCK) do not show MMC-related changes (Konturek *et al.* 1986; Lee *et al.* 1986; Qvist *et al.* 1990). The existence of gastrin concentration fluctuations is controversial and is probably secondary to the periodic

secretion of gastric juice (Keane *et al.* 1980; Konturek *et al.* 1986). In pigs, Cuber *et al.* (1985, 1986) reported apparent fluctuations of plasma PP, motilin, somatostatin, secretin, gastrin and CCK.

The GI motility and the exocrine pancreas periodic activities of young ruminants have been described in detail previously (Ruckebusch & Bueno, 1973; Dardillat, 1977; Dardillat & Marrero, 1977; Zabielski *et al.* 1993, 1995). In brief, the small-intestinal MMC consists of three distinct phases in calves: no spiking activity (NSA) phase; irregular spiking activity (ISA) phase, and regular spiking activity (RSA) phase (Dardillat & Ruckebusch, 1973). The NSA phase is electromyographically characterized as a period of quiescence (no spike bursts, slow waves only) which results in a lack of mixing and propulsive contractions and no

digesta flow in the examined segment of the intestine (Dardillat, 1977). During the ISA phase the frequency and amplitude of spike bursts increase in an irregular manner, and these reach their maxima during the RSA phase. The mixing contractions dominate during the early ISA phase, and so do the propulsive contractions during the last ISA and RSA phases. The GI tract secretory activities coincide with the phases of duodenal MMC, e.g. the secretion of pancreatic juice is low during the NSA phase, and high during the ISA phase (Zabielski *et al.* 1993). In contrast to single-stomached animals in which feeding disrupts the periodic activity for several hours (Code & Marlett, 1975; Konturek *et al.* 1986), in preruminant and ruminant calves the periodic activity of the abomasum, small intestine and pancreas is transiently influenced by feed (Ruckebusch & Bueno, 1973; Girard & Sissons, 1992; Zabielski *et al.* 1997). Therefore it has been suggested that the periodic activity of the GI tract in ruminants is actively involved in the digestive processes. In preruminant calves, plasma secretin (but not CCK) oscillations in phase with the duodenal MMC and periodic pancreatic secretion were demonstrated (Zabielski *et al.* 1994). The authors suggested that secretin pulses would modulate gastric motility and pancreatic secretory function, but would not be important in the generation of their periodic activities. No data concerning rhythmic variations of the other major gut regulatory peptides in preruminant calves are currently available. Therefore the aim of the present study was, first, to clarify whether plasma concentrations of several major gut regulatory peptides (PP, motilin, gastrin, CCK, secretin, vasoactive intestinal polypeptide (VIP), and somatostatin) fluctuate in phase with the duodenal MMC. Second, we investigated whether these relations could be modified by

different sources of dietary protein. Skimmed-milk protein, retaining clotting activity in the abomasum, and fish protein devoid of this activity, which produced different patterns of gastric emptying and intestinal flow of digesta (Guilloteau *et al.* 1975, 1981, 1986b) were compared in order to investigate the influence of dietary protein on rhythmic activity of gut peptides and gastrointestinal motility.

Materials and methods

Treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals. Four Holstein–Friesian male preruminant calves (3 weeks old) surgically implanted with three NiCr wire bipolar electrodes on the duodenum (Dardillat & Ruckebusch, 1973), and a catheter in the external jugular vein were used. After 1 week postsurgical recovery the calves were randomly divided into two groups which received milk diet (MD) or fish diet (FD). The experiments were performed twice on each animal with 1 week rest between the experiments. After that, the diet was switched, and following a 1-week adaptation period the experiments were repeated. The food was given at 12 h intervals at 08.00 hours and at 20.00 hours. The composition of the diet is shown in Table 1.

Before the experiment the indwelling electrodes were connected to an eight-channel electroencephalograph (mini 8 Alvar, Alvar Electronic, Paris, France) equipped with a time constant of 0·3 s. Tracings were made at a paper speed of 15 mm/min. The three phases of duodenal activity (NSA, ISA, and RSA) were identified according to Dardillat & Ruckebusch (1973). Recordings started 1–2 h

Table 1. Composition of the milk replacers based on either skimmed-milk powder (MD) or fish-protein concentrate (FD)

	MD	FD
Ingredients (g/kg powder)		
Source of protein	681*	208†
Tallow‡	175	164
Whey powder	—	469§
Lactose	92	84
Starch	45	62
DL-Methionine	1·7	2·4
Lysine hydrochloride	—	2·7
$CaH_2(PO_4)_2 \cdot H_2O$	1·0	3·2
$MgSO_4$	0·34	0·45
NaCl	0·30	—
Trace elements, vitamins, antibiotics‖	4	4
Composition (g/kg DM)		
Protein	246	239
Fat	205	193
Ash	66	76

* Spray-dried skimmed-milk powder.
† CPSP 90′, partially-hydrolysed white-fish protein concentrate; Coopérative de Traitement des Produits de la Pêche, Boulogne-sur-Mer, France.
‡ Tallow homogenized into concentrated skimmed-milk (MD) or whey (FD) before spray-drying (350 g fat/kg DM).
§ Sweet whey powder (135 g N × 6·25/kg DM).
‖ Providing (mg/kg diet): $CuSO_4.5H_2O$ 25, $ZnSO_4.H_2O$ 230, $MnSO_4.H_2O$ 125, KI 6, $CoSO_4.7H_2O$ 2·5, retinol 4·13, cholecalciferol 0·15, DL-α-tocopheryl acetate 100, menadione 2·0, thiamin 6·4, riboflavin 3·0, pyridoxine 7·2, cyanocobalamin 0·05, ascorbic acid 100, pantothenic acid 2·2, biotin 0·75, nicotinic acid 2·2, pteroylmonoglutamic acid 1·0, choline 780, betaine 500, furazolidone 50, bacitracine 50.

before morning feeding and were continued to record eight postprandial MMC cycles. Food was given at about 08.00 hours; the moment of feeding was assigned by the MMC recording: feeding started during the late NSA phase or the early ISA phase, which appeared at 08.00 hours ± 20 min. Blood was withdrawn in the preprandial and postprandial periods at least once during NSA and RSA phases of the duodenal MMC. During the ISA phase, blood samples were taken at least twice: during early ISA (ISA1) and late ISA (ISA2) phases. During the ISA phase of the prandial period and the first postprandial cycle, blood was withdrawn every 15–20 min. Blood samples were collected in tubes containing heparin (500 IU/ml; Choay Heparin, Sanofi Winthrop, France) and aprotinin (10 000 IU/ml; Iniprol, Sanofi Winthrop). Plasma concentrations of seven peptides (PP, motilin, secretin, VIP, gastrin, CCK, somatostatin) were measured by radioimmunoassay using a double antibody technique (for assay details see Table 2). Gut regulatory peptide concentrations are expressed as pg/ml plasma and shown as mean values with their standard errors. The repeated measures ANOVA followed by the Tukey–Kramer multiple comparisons test was used for comparisons between phases within the MMC cycle and between respective phases taken from subsequent MMC cycles, and Student's t test for comparisons between the two diets (InStat for Macintosh, v. 2.03, GraphPad Software, San Diego, CA, USA). Duration of MMC was expressed in min and evaluated statistically using the Kruskall–Wallis non-parametric ANOVA followed by Dunn's multiple comparisons test for comparison within one diet, and Student's t test for comparison between the two diets. In all statistical analyses $P < 0.05$ was taken as the level of significance.

Results

Duodenal migrating myoelectric complex

A three-phased MMC pattern in the GI tract was recorded in the experimental calves. The MMC cycles differed, however, between the calves fed on MD and those fed on FD (Fig. 1, Table 3). In MD calves the mean duration of the preprandial MMC cycles was 44·7 (SEM 4·5) min. Feeding significantly prolonged the MMC cycle in which food was administered (89·4 (SEM 9·6) min; non-parametric ANOVA followed by Dunn's post-test; $P < 0.001$). The

duration of postprandial MMC cycles, however, did not differ significantly from that of the preprandial cycles.

In FD calves, the duration of preprandial MMC cycles was 26·5 (SEM 2·6) min. As compared with MD calves, a significant shortening (paired t test; $P < 0.01$) of preprandial MMC cycles in FD calves was the result of shortening both the NSA phase and the ISA phase (Table 3). The mean duration of the prandial MMC cycle in FD calves was 117 (SEM 11.8) min, and it was significantly longer (nonparametric ANOVA followed by Dunn's post-test; $P < 0.001$) as compared with preprandial MMC. The RSA phase of prandial MMC was of shorter duration as compared with preprandial cycles (Table 3). The duration of the first postprandial MMC cycle and the following MMC cycles in FD calves did not differ significantly from the preprandial MMC due to high variability of the postprandial values (Fig. 1, Table 3).

Plasma pancreatic polypeptide and motilin

The preprandial plasma concentration of PP apparently fluctuated in phase with the duodenal MMC in MD calves (Fig. 2): the PP concentration was low during NSA and increased during ISA (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.01$). During the prandial MMC and the first postprandial cycle these fluctuations disappeared. In the following second to fourth postprandial cycles, the concentration of PP during ISA and RSA was lower than preprandial values (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.01$). The concentration of PP during NSA did not change significantly. In the fifth to the eighth MMC cycles, the PP concentration gradually increased and the fluctuations in phase with duodenal MMC reappeared.

There were no significant differences in the preprandial PP concentrations between MD and FD calves. In FD calves, the observed preprandial fluctuations in phase with duodenal MMC were statistically not significant due to high variability of data (Fig. 2). Following a fish meal, the concentration of PP showed a biphasic pattern; a slight temporary increasing tendency at the beginning of the prandial cycle (NS) was followed by a decrease that was significant in the first to fourth postprandial MMC cycles (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.05$). As in MD calves, in FD calves the

Table 2. Characteristics of radioimmunoassay of gut regulatory peptides

| Peptide...	PP	Motilin	Secretin	VIP	Gastrin	CCK	Somatostatin
Reference	Miazza *et al.* (1985)	Cuber *et al.* (1985)	Pelletier *et al.* (1978)	Chayvialle *et al.* (1980)	Chayvialle *et al.* (1978)	Miazza *et al.* (1985)	Chayvialle *et al.* (1980)
Antiserum	26B	21D	32K	76E	28E	67H	56D
Standard	Bovine PP	Porcine motilin	Porcine secretin	Porcine VIP	Human gastrin I (1–17 nonsulfated)	Porcine CCK-33	Ovine somatostatin (1–14)
Reactivity with other peptides (non-specific)	<1 % NPY and PYY	~0	<5 %	<1 %	4 % CCK-33	1 % gastrin	100 % S28
Inter-assay variation (%)	9·0	11·3	6·2	7·2	13·4	9·0	12·3
Intra-assay variation (%)	6·0	5·9	5·8	7·0	9·3	13·5	7·1
Recovery (%)	80	75	87	79–100	75–94	62–80	80–98

CCK, cholecystokinin; NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, polypeptide YY; S, somatostatin; VIP, vasoactive intestinal polypeptide.

R. Zabielski *et al.*

Table 3. Duration of duodenal migrating myoelectric complex (MMC) phases in calves fed with milk replacer based on skimmed-milk powder (MD) or fish-protein concentrate (FD)

(Mean values with their standard errors for four calves; ranges are given in parentheses)

Phase	Preprandial Mean	SE	Prandial Mean	SE	Postprandial 1st Mean	SE	2nd Mean	SE	3rd Mean	SE	4th Mean	SE	5th Mean	SE	6th–8th Mean	SE	P value in row
MD																	
NSA	10·2 (7–15)	0·9			8·0 (5–15)	1·0	10·6 (5–17)	1·1	10·5 (6–13)	0·9	11·9 (0–17)	2·0	10·8 (9–16)	1·7	12·5 (0–21)	1·6	0·372
ISA	32·4^a (17–44)	5·1	87·1^b (41–131)	9·7	37·4^{ab} (11–61)	4·8	38·8^{ab} (9–68)	5·8	42·7^{ab} (27–55)	3·0	33·1^a (14–64)	4·2	32·1^a (14–70)	5·6	23·3^a (12–58)	5·7	0·0001
RSA	2·9 (2–4)	0·2	2·3 (2–3)	0·1	2·2 (1–3)	0·2	2·5 (2–3)	0·2	2·1 (1–3)	0·1	2·4 (1–3)	0·2	2·6 (1–4)	0·3	2·4 (2–3)	0·3	0·085
FD																	
NSA	6·6** (3–14)	0·8			16·1 (0–58)	6·7	14·7 (4–45)	4·0	12·0 (6–18)	1·5	12·1 (2–17)	1·6	10·9 (7–21)	1·6	11·0 (4–45)	1·7	0·0915
ISA	16·5^a** (7–28)	2·4	115·2^b (64–185)	11·8	36·3^{ab} (0–145)	13·0	27·3^a (0–61)	6·1	32·5^{ab} (11–92)	7·3	31·3^a (5–76)	7·5	20·5^a (0–39)	4·0	27·4^a (0–48)	6·7	0·0003
RSA	3·4^a (2–5)	0·3	2·2^b (2–3)	0·1	2·4^a (2–3.5)	0·2	2·4^a (1.5–3)	0·2	2·5^a (2–4)	0·2	2·3^a (1.5–3)	0·2	2·8^a (2–4)	0·2	2·4^a (2–5)	0·2	0·0027

NSA, no spiking activity; ISA, irregular spiking activity; RSA, regular spiking activity; phase of the duodenal MMC.

^{a,b} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$ (Kruskall–Wallis non-parametric ANOVA followed by Dunn's multiple comparison test).

Mean values were significantly different from those for MD calves for the corresponding variable, ** $P < 0.01$ (paired t test).
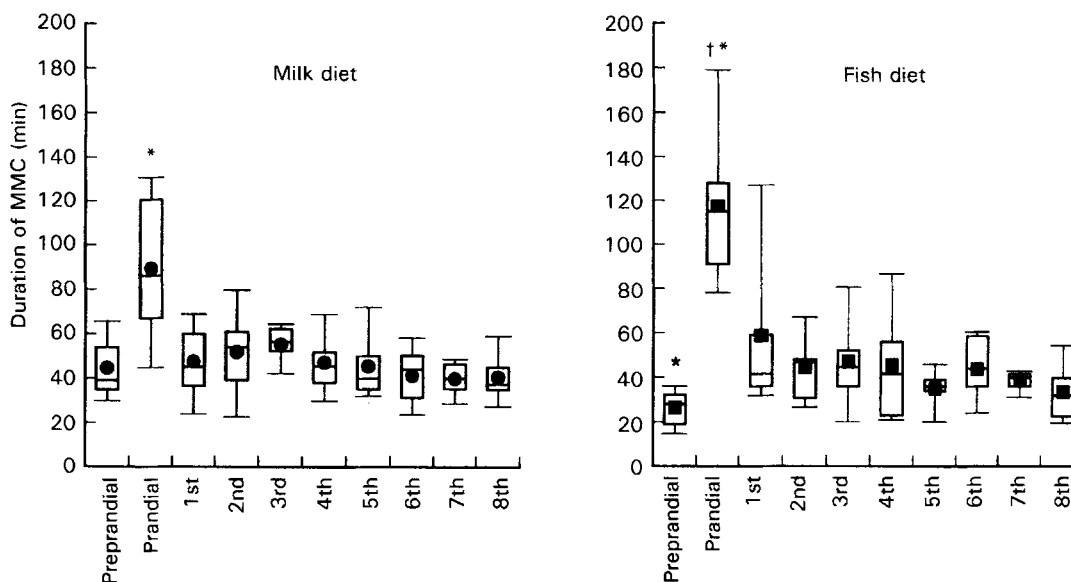
Fig. 1. Duration of the duodenal migrating myoelectric complex (MMC) in preruminant calves fed on a milk diet (●) or a fish diet (■). Values are arithmetic means (●, ■) for four calves, with minimum and maximum values indicated by vertical bars and the median value depicted by an open bar with a horizontal line. Mean values were significantly different from that for the preprandial cycle in the milk diet, * $P < 0.001$ (Kruskall–Wallis non-parametric ANOVA followed by Dunn's multiple comparison test for comparison data from calves fed on the milk diet, and unpaired t test for comparison data from calves fed on the fish diet). Mean values were significantly different from that for the preprandial cycle in the fish diet, † $P < 0.001$ (Kruskall–Wallis non-parametric ANOVA followed by Dunn's multiple comparison test).

decrease in PP concerned the ISA and RSA phases but not the NSA phase. In FD calves, the concentration of PP gradually increased during the fifth to eighth cycles of the MMC, and significant fluctuations in phase (low PP concentration during NSA and high PP concentration during ISA) were observed in the last postprandial MMC cycles (sixth to eighth MMC). In FD calves the PP concentration returned to preprandial values in the sixth to eighth MMC cycles.

Preprandial plasma motilin concentrations in MD calves were between 28·3 (SEM 6·3) pg/ml and 36·5 (SEM 5·9) pg/ml during early ISA, NSA and RSA phases, and peaked during the late ISA phase (50·5 (SEM 6·9) pg/ml; repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.05$). This pattern, however, was not observed in the postprandial period. There were no other changes in the postprandial motilin concentrations as compared with preprandial ones. In FD calves, no motilin fluctuations
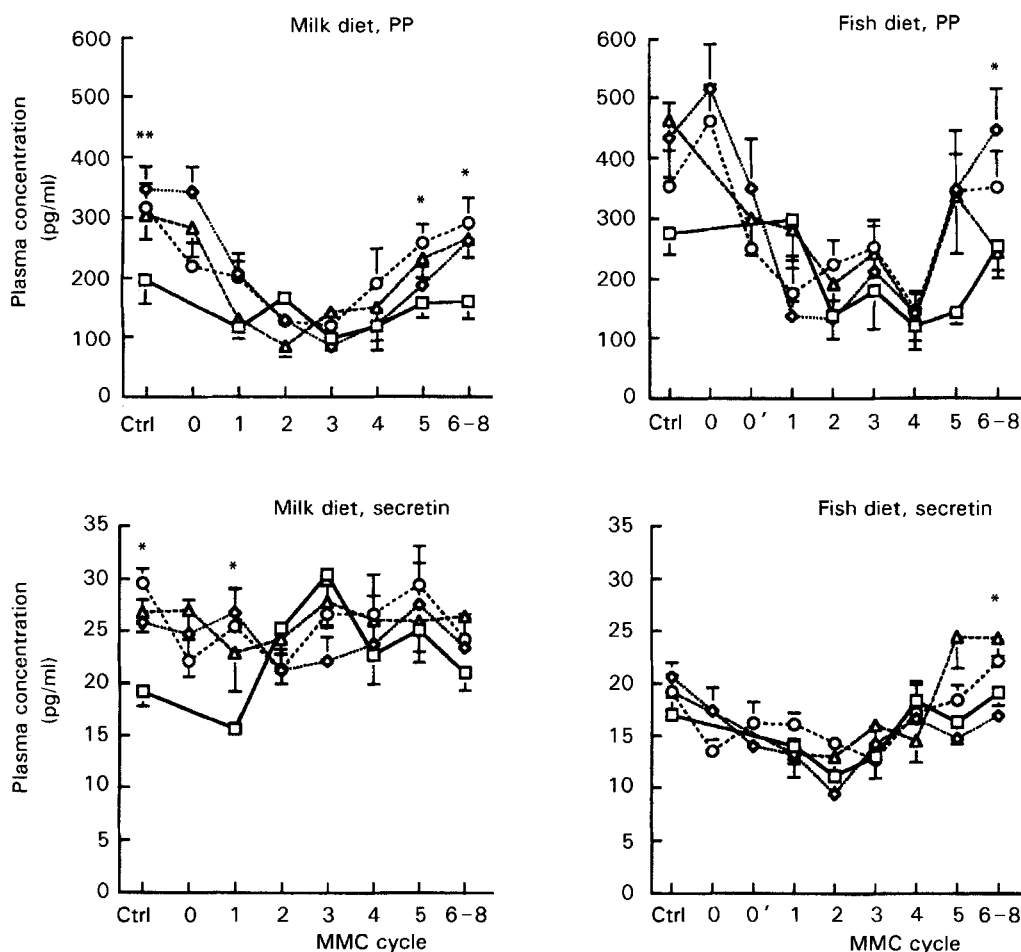
Fig. 2. Plasma concentrations of pancreatic polypeptide (PP) and secretin in phases of the preprandial (Ctrl), prandial (0 and 0') and postprandial (1,2, ... and 6–8) duodenal migrating myoelectric complexes (MMC) in preruminant calves fed on a milk diet or a fish diet. (□), No spiking activity phase; (◇), early irregular spiking activity (ISA1) phase; (○), late irregular spiking activity (ISA2) phase; (△), regular spiking activity phase. In calves fed on the fish diet, blood samples collected during the prandial ISA phase, due to its long duration, were divided into four consecutive periods (0-ISA1, 0-ISA2, 0'-ISA1, 0'-ISA2) in order to express better the kinetics of gut regulatory peptide concentrations in this period. Extreme values in the cycle were significantly different: $*P<0.05$, $**P<0.01$ (repeated measures ANOVA followed by Tukey–Kramer multiple comparisons test).

were observed in either the preprandial or the postprandial period, and motilin concentration varied between 28·1 (SEM 4·1) pg/ml during ISA and 34·7 (SEM 4·3) during RSA phases (NS) (results not shown).

Plasma secretin and vasoactive intestinal polypeptide

In MD calves, the periodic fluctuations of plasma secretin concentration were observed during the preprandial MMC cycles and during the first postprandial MMC (the concentration of secretin during the ISA phase was about 1·5-fold higher than during the NSA). After that, the fluctuations of secretin concentration were not observed since it markedly increased in the following postprandial NSA phases (Fig. 2).
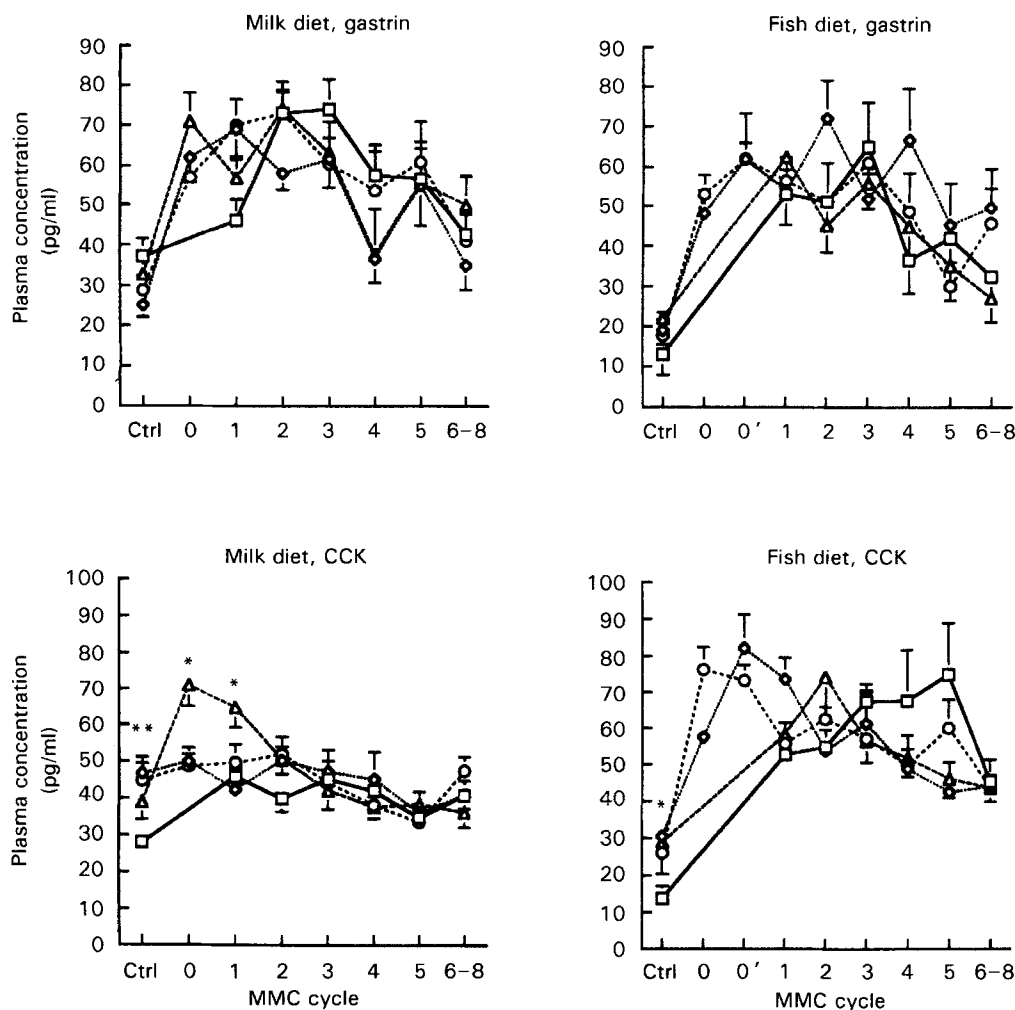
In FD calves the preprandial concentration of secretin was significantly lower, as compared with MD calves, during the late ISA (ISA2; paired t test; $P < 0.01$) and RSA (paired t test; $P < 0.05$) phases. In FD calves, no significant periodic fluctuations were observed before or just after feeding, whilst during the sixth to eighth MMC cycles the fluctuations were present (Fig. 2). However, the minimum

value was recorded during the early ISA phases and the peak during RSA phases (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.05$). The concentration of plasma secretin significantly decreased in MMC cycles 1–3 following feeding with FD; this effect mainly concerned the concentration during the early ISA phase (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.05$).

No periodic fluctuations were observed in plasma VIP in MD and FD calves either before or after feeding. The concentration of plasma VIP was between 23·2 (SEM 4·1) pg/ml during ISA1 and 26·1 (SEM 3·8) pg/ml during ISA2. In both groups of calves, feeding caused no significant changes in plasma VIP, although in the postprandial period plasma VIP tended to decrease in NSA, early ISA and RSA phases by approximately 15 % (NS).

Plasma gastrin and cholecystokinin

Preprandial and postprandial concentrations of gastrin in blood plasma did not show significant fluctuations in MD

Fig. 3. Plasma concentrations of gastrin and cholecystokinin (CCK) in phases of the preprandial (Ctrl), prandial (0 and 0′) and postprandial (1, 2, ... and 6–8) duodenal migrating myoelectric complexes (MMC) in preruminant calves fed on a milk diet or a fish diet. (□), No spiking activity phase; (◊), early irregular spiking activity phase; (○), late irregular spiking activity phase; (△), regular spiking activity phase. For further details, see Fig. 2. Extreme values in the cycle were significantly different: * $P < 0.05$, ** $P < 0.01$ (repeated measures ANOVA followed by Tukey–Kramer multiple comparisons test).

calves, although a tendency toward a higher gastrin concentration in the NSA phase than in the ISA1 phase could be observed in preprandial and some postprandial MMC cycles (NS; Fig. 3). Plasma gastrin concentration markedly increased after feeding (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.01$). The increase started in the prandial cycle and continued for five MMC cycles.

FD significantly decreased the interdigestive plasma gastrin concentration during NSA (paired t test; $P < 0.05$) as compared with MD. ISA and RSA gastrin concentrations showed tendencies to decrease (NS; Fig. 3). As in MD calves, in FD calves neither preprandial nor postprandial concentrations of plasma gastrin showed any periodic fluctuations in phase with duodenal MMC. Plasma gastrin significantly increased following FD (repeated measures ANOVA followed by Tukey–Kramer post-test; $P < 0.05$) to the values observed following MD.

Preprandial plasma CCK fluctuated in phase with MMC in MD calves (nadir during NSA and peak during ISA1 and ISA2 phases). In the prandial cycle and the first postprandial cycles the peak shifted to the RSA phase,

and in the subsequent cycles the fluctuations were no longer observed since plasma CCK concentrations in postprandial NSA increased up to corresponding ISA and that in RSA decreased (Fig. 3). Plasma CCK during the ISA phases remained unchanged following feeding.

In FD calves, preprandial plasma CCK was lower than in MD calves (paired t test; $P < 0.05$), and the rate of plasma CCK periodic fluctuations in phase with duodenal MMC was slightly higher (2-fold in FD *v.* 1·5-fold in MD calves). Following feeding, the fluctuations in plasma CCK were no longer observed over the entire collection period. Feeding caused a significant increase in plasma CCK; the increase was 4·5-fold during NSA phases, and 2-fold during ISA and RSA phases (Fig. 4). The incremental increases in plasma CCK and gastrin in response to food (prandial and postprandial cycles 1–8) were significantly higher in FD than in MD calves (unpaired t test, $P < 0.05$).

Plasma somatostatin

In milk-fed calves, plasma somatostatin concentration fluctuated in phase with duodenal MMC, showing a nadir
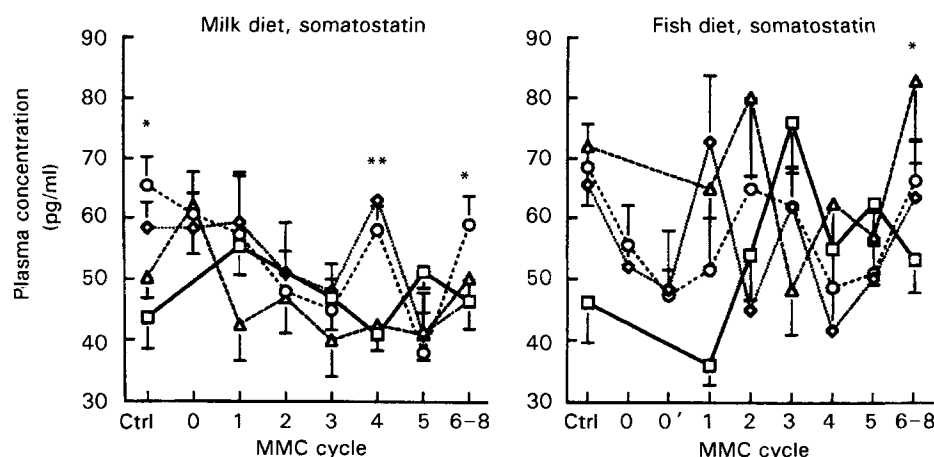
Fig. 4. Plasma concentration of somatostatin in phases of the preprandial (Ctrl), prandial (0 and 0′) and postprandial (1, 2, ... and 6–8) duodenal migrating myoelectric complexes (MMC) in preruminant calves fed on a milk diet or a fish diet. (□), No spiking activity phase; (◇), early irregular spiking activity phase; (○), late irregular spiking activity phase; (△), regular spiking activity phase. For further details, see Fig. 2. Extreme values in the cycle were significantly different: * $P < 0.05$, ** $P < 0.01$ (repeated measures ANOVA followed by Tukey–Kramer multiple comparisons test).

during NSA and a peak during late ISA (Fig. 4). This pattern was observed in the preprandial and in the late postprandial period (Fig. 4, 6–8th MMC cycles after feeding). In the early postprandial period (cycles 1–3) the somatostatin peak during the last ISA was not observed. Feeding did not produce significant modifications of somatostatin concentration during the NSA, early ISA and RSA phases.

In FD calves, the preprandial fluctuations of somatostatin were not significant (repeated measures ANOVA followed by Tukey–Kramer post-test; $P = 0.087$) due to high scattering of the data, and the peaks shifted from late ISA to RSA phases (Fig. 4). These tendencies were not observed in postprandial MMC cycles 1–5, since the somatostatin peak during the RSA diminished. In the postprandial cycles, the concentration of somatostatin tended to be higher in FD calves than in MD calves (NS).

Discussion

In preruminant calves fed on MD, the duration of MMC cycles did not differ from that reported previously (Ruckebusch & Bueno, 1973; Dardillat, 1977), and plasma concentrations of PP, motilin, CCK, secretin and somatostatin apparently fluctuated in phase with preprandial MMC. No fluctuations in plasma gastrin and VIP were observed. Feed affected these fluctuations in a specimen-specific manner. The present results confirm fluctuations of secretin reported previously (Zabielski et al. 1994) and reveal certain similarities between the preruminant calves and single-stomached animals with regard to PP and motilin (Konturek et al. 1986; Lee et al. 1986). Except for plasma gastrin, our results corroborate previous findings in pigs (Cuber et al. 1985, 1986). However, in preruminant calves fed on FD, both MMC and gut regulatory peptide physiological fluctuations were noticeably distorted: oscillations in PP and somatostatin concentrations were non-significant due to the large variability of the data; and those

of motilin and secretin were apparently abolished. The present study clearly demonstrates that the source of dietary protein has an impact on the GI endocrine function.

Preprandial fluctuations in plasma PP and motilin concentration in MD calves were of similar amplitude to those observed in dogs (Keane et al. 1980; Chen et al. 1983; Konturek et al. 1986). In the calves, however, the peak of plasma PP concerned the early ISA phase rather than the late ISA phase, and the motilin peak was observed during the late ISA phase. Duodenal MMC cycles were recorded following feeding in calves, whereas PP cycles disappeared for four subsequent postprandial MMC cycles, and motilin cycles were not seen postprandially. Moreover, unlike in single-stomached species, plasma PP markedly decreased following feeding. This unique pattern has been observed previously (Toullec et al. 1992) and it may be species-specific. Assuming that plasma PP reflects the vagal tone in the calf, as has been proven for dogs and human subjects (Schwartz, 1983), then the postprandial decrease of plasma PP to the level of the preprandial nadirs would indicate a diminished vagal tone in the early postprandial state. This supposition may explain postprandial reduction of pancreatic juice secretion observed in preruminant calves (Le Dréan et al. 1997) since it has been shown that this secretion depends largely on the vagal activity (Pierzynowski et al. 1992; Zabielski et al. 1993). The physiological relevance of PP and motilin in calves needs to be clarified, although the relevance of motilin in regulation of sheep MMC has been questioned (Plaza et al. 1996). After replacing milk with fish protein, the fluctuations in PP concentration were significant in the late postprandial period, and were less clear due to a high scattering of the data during the preprandial period while motilin fluctuations were not detected at all. Presumably the pattern abomasal emptying rate and intestinal digesta flow concomitant with different stimulation of pancreatic function may be responsible for these discrepancies. In studies on dogs with the autotransplanted pancreas, a coexistence of interdigestive MMC and motilin cycles but

not PP cycles was found (Zimmerman *et al.* 1992). Similarly, in human subjects with impaired pancreatic function (chronic pancreatitis), plasma PP and pancreatic juice cycles were not coordinated with the interdigestive antroduodenal motility (Pieramico *et al.* 1995). Thus, it is suggested that the synchrony between plasma PP and motilin concentrations and duodenal and pancreatic cycles may be discontinued by some gastrointestinal disorders as well as by 'unphysiological' feed (e.g. containing protein devoid of clotting activity in the abomasum and resulting in a massive inflow of digesta into the proximal duodenum immediately after feeding in preruminant calves).

Fluctuations in plasma secretin were present in milk-fed calves and in the late postprandial period in calves fed on FD. Thus, our results indicate that both increased and reduced duodenal digesta flow may disorganize these fluctuations. The lack of VIP cycles in the peripheral circulation is rather unsurprising. VIP is known to be a neuromediator that is released locally by nerve terminals and is not followed by detectable changes in the general circulation under physiological conditions (Reid *et al.* 1988). However, in some pathological situations (severe diarrhoea, GI parasite invasion) the plasma concentration of VIP may increase significantly (Dockray, 1994).

In contrast to previous findings (Zabielski *et al.* 1994), fluctuations in plasma CCK concentration were found in the present study. One explanation could be the difference in milk replacer formula, i.e. the presence of soyabean protein used in the previous study (Zabielski *et al.* 1994). It has been shown that milk replacer containing soyabean protein dramatically elevates plasma CCK as compared with milk, which might nullify physiological oscillations (Guilloteau *et al.* 1986*a*). Interestingly, apart from an increase of plasma CCK during the RSA there was no postprandial increase following MD diet. This finding contrasts with previous reports, including ours (Guilloteau *et al.* 1986*a*), and implies that physiological periodic activity must be taken into account in GI studies. Plasma gastrin did not fluctuate in experimental calves, and feeding caused a several-fold increase of plasma gastrin following both diets. Nevertheless, significantly lower preprandial concentrations of plasma gastrin and CCK were found with FD as compared with MD. This result may be related to the abomasal emptying rate, which is faster soon after feeding and minimal after that, and it may have negative consequences in the development of the GI tract (Guilloteau *et al.* 1997). Circulating gastrin was shown to be a major regulator of oxyntic mucosal growth, and an important stimulator of gastric endocrine cells and intestinal mucosa growth (Walsh, 1994). It also seems to be an important trophic factor for the pancreas in calves (Le Meuth *et al.* 1993). CCK has been proven to stimulate growth of the pancreas in several animal species (Liddle, 1994), and to reduce bacterial overgrowth and translocation in the small intestine (Wang *et al.* 1996).

The question of whether the fluctuations observed in the present calves are the cause or the effect of the periodic activities of the upper GI tract should be addressed. In fasted dogs PP and motilin cycles were independent of fluctuations of gastric and pancreatic secretions (Chen *et al.* 1983; Lee *et al.* 1986). Two arguments arising from the

present study suggest that, in calves also, the fluctuations of examined peptides in plasma may not be the cause of the GI MMC cycles. First, in MD calves after feeding the fluctuations of gut regulatory peptides disappeared for several hours, whereas the MMC cycles did not. In addition, the periodic pancreatic cycles were disrupted only for about 1 h by feeding and became visible thereafter (Zabielski *et al.* 1993, 1997). Moreover, intravenous continuous infusion of CCK and motilin, which increased several-fold their plasma concentrations (and apparently disrupted their physiological fluctuations in the peripheral blood), did not abolish duodenal and pancreatic cycles (Zabielski *et al.* 1995). In the same study, administration of exogeneous secretin disrupted the duodenal MMC but not the pancreatic cycles. This suggests that the observed fluctuations of gut regulatory peptides are not the cause of GI motor or secretory cycles. The second argument is that, after replacing milk protein by fish protein, only fluctuations of plasma CCK and somatostatin concentrations were manifested in the preprandial period. The diet based on fish protein devoid of clotting activity modifies the abomasal emptying, small-intestinal MMC, and the passage speed of the digesta. It is known to cause rapid digesta flow into the duodenum soon after feeding and a minimal flow after that (Guilloteau *et al.* 1975, 1981, 1986*b*). In these circumstances the upper GI tract during the preprandial period would be nearly empty, a situation that is not observed in preruminant calves fed on cow's milk, or milk replacer based on skimmed-milk powder. Lack of preprandial and early postprandial secretin cycles in calves fed on non-clotting protein, supports the idea that fluctuations of plasma secretin are secondary and may be related to changes in duodenal pH. In summary, it seems, therefore, that the fluctuations of PP, motilin, secretin, and somatostatin concentrations are the result of a temperate digesta passage that is present in milk-fed calves several hours after feeding.

It could be thought that the regularity of GI rhythms reflects animal well-being, since such patterns were observed in animals that were healthy, well fed, unstressed, and well-accustomed to experimental conditions (Code & Marlett, 1975). The experimental calves fed on MD showed the MMC pattern similar to that normally observed in preruminant calves (Ruckebusch & Bueno, 1973; Dardillat, 1977). However, feeding with non-clotting milk replacer impaired the GI motility, shortened the preprandial MMC, prolonged the duration of prandial MMC, and caused remarkable irregularity of the postprandial MMC cycles. Therefore it is probable, although not examined in this study, that the passage, mixing and absorption of the GI content (Ruckebusch, 1970; Fioramonti *et al.* 1982; Girard & Sissons, 1992) were different in FD calves as compared with MD calves. Consequently, we suggest that the overall changes in plasma gut peptides observed in FD calves (lack of fluctuations of PP, motilin and secretin concentrations, lowered interdigestive gastrin, CCK and secretin concentrations, and high scattering of the data in most of the peptides examined) reflect impairment of the endocrine function caused by feed. Differences in chemical composition (e.g. hydrolysed dietary proteins with FD or amino acid composition which is less adequate to cover the

requirements in preruminant calves with fish protein than with milk protein; Toullec, 1989) and in other digesta variables reported earlier (e.g. pH of digesta; Guilloteau et al. 1975; products of enzymic hydrolysis; Guilloteau, 1986) may also affect the gut regulatory peptide response.

In conclusion, periodic fluctuations of the plasma gut regulatory peptides in concert with the GI MMC were observed in preruminant calves. Periodic fluctuations of the peptides transiently disappeared following feeding with a milk-based diet. Feeding with fish protein markedly disturbed both the periodic fluctuations of the majority of examined gut peptides and the small-intestinal MMC. The observed fluctuations of plasma gut regulatory peptides seem to be secondary to GI motility and secretory cycles, although monitoring these fluctuations might be considered a sensitive diagnostic tool for examination of animal well-being in nutritional and behavioural studies.

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