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Original article

## Small intestinal motility disorders in preruminant calves chronically fed a diet based on antigenic soya: characterization and possible mediators

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**Abstract** – Intestinal motility disorders and some mediators implicated in these disorders were studied in preruminant calves that had been chronically fed a diet containing an antigenic heated soyabean flour (HSF) for 3 months. The calves in the present study had previously been shown to present strong immune reactions against soyabean proteins, as assessed through plasma antibody titres, direct skin tests and *in vitro* lymphoproliferation. Four of these calves sensitive to soya were fitted with an abomasal catheter and wire electrodes on the jejunum. Myoelectric activity was recorded over 7 h following test meals containing skim milk powder (SMP), HSF or a non-antigenic hydrolysed soya protein isolate (HSPI). The pattern of myoelectric complexes migrating to the jejunum was regular with SMP (mean durations of phases I, II and III: 26, 38 and 5.28 min, respectively). With HSF, diarrhoea appeared, and the total duration of phase I decreased from 149 to 68 min ( $P < 0.01$ ) while that of phase II increased from 239 to 328 min ( $P < 0.01$ ). The mean duration of phase III decreased from 5.3 to 3.9 min ( $P < 0.01$ ). These changes, including diarrhoea, were substantially reversed by feeding HSPI. When promethazine, a H-1 histamine receptor antagonist, was administered *i.v.* prior to feeding HSF the number of phases I tended to decrease and diarrhoea virtually disappeared. In contrast, indomethacin, a cyclooxygenase inhibitor, had limited effects on motility patterns and diarrhoea. These disorders were partially reproduced by *i.p.* administration of platelet-activating factor (PAF) prior to feeding with SMP. These findings suggest that calves chronically fed antigenic soya suffer from immune-mediated motility disorders which are linked to histamine action via H-1 receptors, and possibly with PAF. The role of arachidonic acid catabolites of the cyclooxygenase pathway is probably minor.  
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**calf / intestinal motility / hypersensitivity / mediator / soya**

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**Résumé – Perturbations motrices de l'intestin grêle chez le veau préruminant consommant chroniquement un régime à base de soja antigénique : caractérisation et médiateurs potentiels.** Les troubles de la motricité intestinale et certains médiateurs impliqués ont été étudiés chez des veaux préruminants ayant consommé un aliment riche en farine de soja chauffée et antigénique (HSF) pendant 3 mois. Ces veaux présentaient des réactions immunologiques importantes contre les protéines du soja (anticorps spécifiques, tests cutanés, lymphoprolifération in vitro). Quatre des veaux sensibles au soja ont été munis d'un cathéter gastrique et d'électrodes jéjunales. L'activité myoélectrique a été enregistrée pendant 7 h après l'infusion gastrique de repas à base de poudre de lait écrémé (SMP), de HSF ou d'isolat de protéines de soja hydrolysées et non antigéniques (HSPI). Les complexes myoélectriques migrant sur le jéjunum ont été réguliers (durée moyenne des phases I, II et III : 26, 38 et 5,28 min) avec SMP. Avec HSF, les diarrhées ont été fréquentes ; la durée totale des phases I a diminué de 149 à 68 min ( $p < 0,01$ ), alors que celle des phases II a augmenté de 239 à 328 min ( $p < 0,01$ ). La durée moyenne des phases III a diminué de 5,3 à 3,9 min ( $p < 0,01$ ). Ces changements n'ont pas été observés avec HSPI et les diarrhées ont été rares. La prométhazine, antagoniste des récepteurs H-1 de l'histamine, administrée par voie IV avant un repas de HSF, a réduit le nombre de phases I ( $p < 0,01$ ) et les diarrhées ont été rares. En revanche, l'indométhacine, inhibiteur de la cyclo-oxygénase, administré par voie IP, a eu peu d'effets sur les troubles moteurs et les diarrhées. Ceux-ci ont été partiellement reproduits par l'administration par voie IP de facteur d'activation des plaquettes (PAF) avant un repas de SMP. En conclusion, les veaux hypersensibles au soja souffrent de désordres de la motricité intestinale dus à l'histamine via les récepteurs H-1, et peut-être au PAF. Les catabolites cyclo-oxygénés de l'acide arachidonique auraient un rôle mineur. © Inra/Elsevier, Paris

**veau / motricité intestinale / hypersensibilité / médiateur / soja**

## 1. INTRODUCTION

Soya is an important protein source for substituting skim milk powder (SMP) in milk replacers for calves. But the nutritional quality of soya products for the calf depends upon the nature of industrial processing. Inadequately treated products such as heated soyabean flour (HSF), when fed to calves, have led to poor growth performance. In immuno-physiological studies, feeding HSF resulted in the production of high levels of circulating anti-soya antibodies and the development of gut motility disturbances associated with diarrhoea [30]. Changes in motility patterns were characterized by reduced intensity of gastric antral activity and increased velocity of migrating myoelectric complexes (MMCs) along the gut [32]. These changes were in turn responsible for a slow rate of gastric emptying and an acceleration in the passage of digesta through the small intestine. These disturbances in gut physiology have been linked with maldigestion and malabsorption observed in calves sensitized to

HSF [31, 34]. Calves chronically fed HSF also develop specific antibodies and sometimes diarrhoea [17].

The mechanisms underlying motility disturbances in calves fed HSF are obscure, both in terms of possible mediators and links with the immune system. In contrast, rodent models of intestinal anaphylaxis provided evidence that such disorders are mediated primarily by biogenic amines and secondly by arachidonic acid derivatives released from intestinal mast cells bearing IgE antibodies specific for the sensitizing antigens [4]. In the rat, other mediators such as platelet-activating factor (PAF) were demonstrated to interfere with intestinal motility induced by endotoxins [23]. In diarrhoeic patients, the faecal excretion of PAF is associated with an inflammatory state of the gut as in Crohn's disease, but not when diarrhoea results from malabsorption [6].

The aim of the present study was to characterize the intestinal motility disorders, and the possible mediators involved,

in calves chronically fed HSF over 3 months. These calves were shown to be particularly sensitive to soya proteins, as demonstrated by direct skin testing and *in vitro* lymphoproliferation [17].

## 2. MATERIALS AND METHODS

### 2.1. Animals and diets

The calves used in the present study were part of a larger experiment that was aimed at identifying soyabean allergens and immune mechanisms [17]. Briefly, 16 male Holstein calves were obtained at 2 d of age from the Inra herd. They were kept on straw in individual pens until the completion of the work. All the calves were fed a milk replacer diet based on SMP up to 1 month of age. Eight calves (control group) were permanently maintained on this diet while the other eight (soya group) received a milk replacer containing a mixture of HSF and whey (70:30 on a protein basis) over 3 months. The crude protein and fat contents were close to 21.6 and 21 % of the dry matter, respectively, in both diets which had been balanced for nutritional studies [16]. The concentrations of immunoreactive glycinin and  $\beta$ -conglycinin in HSF, as determined by ELISA [38], were 39 and 36 mg·g<sup>-1</sup> crude protein, respectively. The level of intake was fixed at 50 g dry matter per kg metabolic weight and per day, and the calves were bucket-fed twice daily.

### 2.2. Electrophysiological studies

Four calves of the soya group, considered to be highly sensitive to HSF on the basis of frequent diarrhoea, high titres of anti-soya antibodies in plasma and large skin and *in vitro* lymphoproliferation responses to soya proteins, were selected for the present experiment. After completion of the study on immune responses, they were switched to the SMP diet. A few days later, they were anesthetized and, under aseptic conditions, fitted with an abomasal catheter (external and internal diameters of 11.1 and 6.4 mm, respectively) and with two pairs of stainless steel electrodes (diameter 110  $\mu$ m, length 110 cm; Cooner Wire Co, Chatsworth, CA, USA) on the serosal face of the first quarter of the jejunum [12, 32]. Elec-

tromyographic recordings were carried out with an ink-writing polygraph (Grass model 7, Grass Instruments, Quincy, MA, USA). Frequency responses of the polygraph were set up to record electrical events occurring between 10 and 35 Hz. Electrical potentials were preamplified and integrated continuously using a summing integrator (Grass model 7P10). Recordings started 7–10 d after surgery. They were conducted generally twice a week, that is with one control and one antigenic test meal. The former was based on either SMP or a non-antigenic hydrolysed soya protein isolate (HSPI; Lallès et al. [16]) and the latter on HSF with or without premedication. The instantaneous myoelectric activity was recorded shortly before and during a period of 7 h after feeding using a chart speed of 36 mm·min<sup>-1</sup>. Integration was carried out manually later on. The test meals were infused through the abomasal catheter 10–15 min after the appearance of a phase III of myoelectric activity. The effects of promethazine (i.v., 2 mg·kg<sup>-1</sup> in saline), a H-1 histamine receptor antagonist, and indomethacin (i.p., 10 mg·kg<sup>-1</sup> in saline), a cyclooxygenase inhibitor, were also investigated with the HSF diet. The effects of PAF (i.p., 25  $\mu$ g·kg<sup>-1</sup> in saline) were studied with the SMP diet. Premedication took place approximately 30–60 min prior to giving the test meal. The influence of each treatment was evaluated on two occasions at intervals of 2 weeks. Diarrhoea was detected visually.

### 2.3. Calculations and statistical analyses

Manual analysis of individual electrical activities recorded on paper charts over time was carried out to determine the number and mean durations of MMCs, phases I (i.e. quiescence with myoelectric activities occurring for less than 5 % of the time), II (irregular spiking activity) and III (regular spiking activity) according to Code and Marlett [3]. The frequency of MMCs was calculated as a number per hour. The total duration of phases I, II or III was calculated over the recording time as the sum of individual durations of each phase, and was expressed in minutes or on a percentage basis. Such a calculation was adopted to reduce the variability observed for the numbers and mean durations of the different phases. This variability was particularly large with test meals containing HSF. Electrophysiological data were

subjected to analysis of variance using the general linear models procedure of SAS [26], taking into account the effects of calf, repetition of recordings and treatment. When the effect of treatment was significant, difference between means were separated by the Bonferroni test [26].

### 3. RESULTS

#### 3.1. Influence of protein source in the test meal

The pattern of jejunal myoelectric activity of the calves was quite regular following a test meal based on SMP (*figure 1*) with a mean duration of MMCs, and phases I, II and III of 67.8, 26, 38 and 5.28 min, respectively (*table 1*). The total duration of phases I, II and III represented 35.5, 57.0 and 7.5 % of the recording time, respectively (*figure 2*). Diarrhoea was never observed with SMP.

In contrast, feeding the HSF diet modified the previous pattern (*table 1*). The mean duration of MMCs with HSF was 72.3 min and was not statistically different from that with SMP. However, the mean durations of phases I and III decreased (−45 % for phase I,  $P < 0.05$ ; −25 % for phase III,  $P < 0.01$ ) with an increase in

phase II (+50 %, NS). This increase reached significance ( $P < 0.01$ ) when the total duration of phase II over 7 h was considered (*figure 2*). Therefore, phases I, II and III represented 16.1, 78.3 and 5.6 % of the recording time with HSF. Two calves exhibited patterns of myoelectric activity that were completely disrupted for hours (*figure 1*). This probably reflected a higher intestinal sensitivity to HSF. Also in these calves spike bursts of intense electric activity appeared on the jejunum less than 20 min after HSF consumption, and were still observed from time to time up to 100 min post-feeding (*figure 3b*). As a comparison taken from another study carried out in this laboratory, a calf presenting neither diarrhoea nor anti-soya antibodies after 3 months of feeding with HSF did not exhibit such spike bursts, illustrating its intestinal insensitivity to this protein source (*figure 3a*). Diarrhoea was observed in three calves, on five out of eight occasions.

The antigen-specific character of intestinal motor disorders with HSF was demonstrated using a test meal based on non-antigenic HSPI. With this feed, the MMC pattern was nearly normal in comparison with that observed with SMP (*figure 1*). However, the mean durations of

**Table 1.** Influence of the protein source in the test meal on the characteristics of the jejunal myoelectric activity in four calves previously fed antigenic soya for 3 months and each receiving test meal on two different occasions (means  $\pm$  SEM).

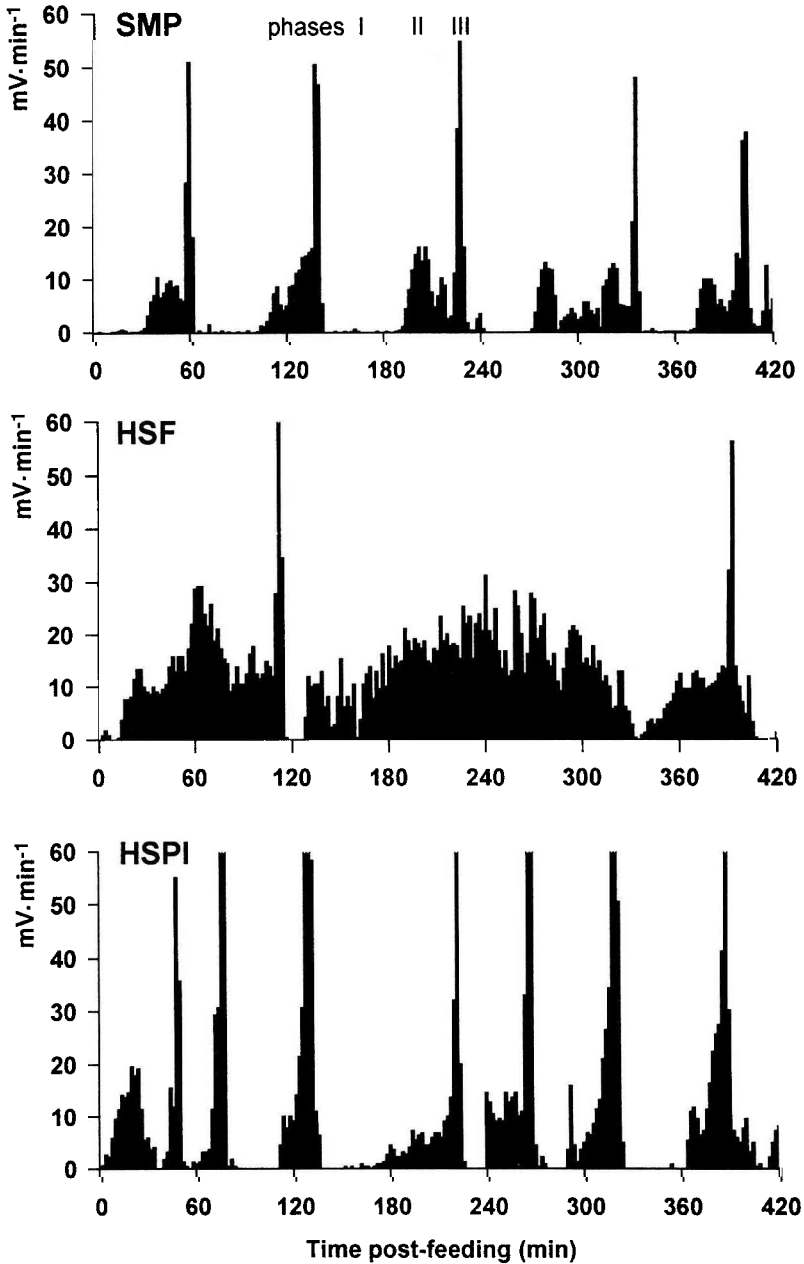
Treatment	Frequency of MMC (n <sup>o</sup> /h)	Mean MMC duration (min)	Mean duration (min) of:			Frequency of diarrhoea
			Phase I**	Phase II*	Phase III**	
SMP	0.90 $\pm$ 0.05	67.8 $\pm$ 3.7	26.0 <sup>a</sup> $\pm$ 3.4	38.0 $\pm$ 1.5	5.28 <sup>Aa</sup> $\pm$ 0.33	0 / 8
HSF	1.04 $\pm$ 0.15	72.3 $\pm$ 12.4	14.4 <sup>b</sup> $\pm$ 1.8	57.0 $\pm$ 11.3	3.91 <sup>Bb</sup> $\pm$ 0.27	5 / 8 (3 calves)
HSPI	0.91 $\pm$ 0.09	73.0 $\pm$ 7.1	20.3 <sup>ab</sup> $\pm$ 3.1	53.9 $\pm$ 8.7	4.42 <sup>b</sup> $\pm$ 0.19	1 / 8

SMP, skim milk powder; HSF, heated soyabean flour; HSPI, hydrolysed soya protein isolate.

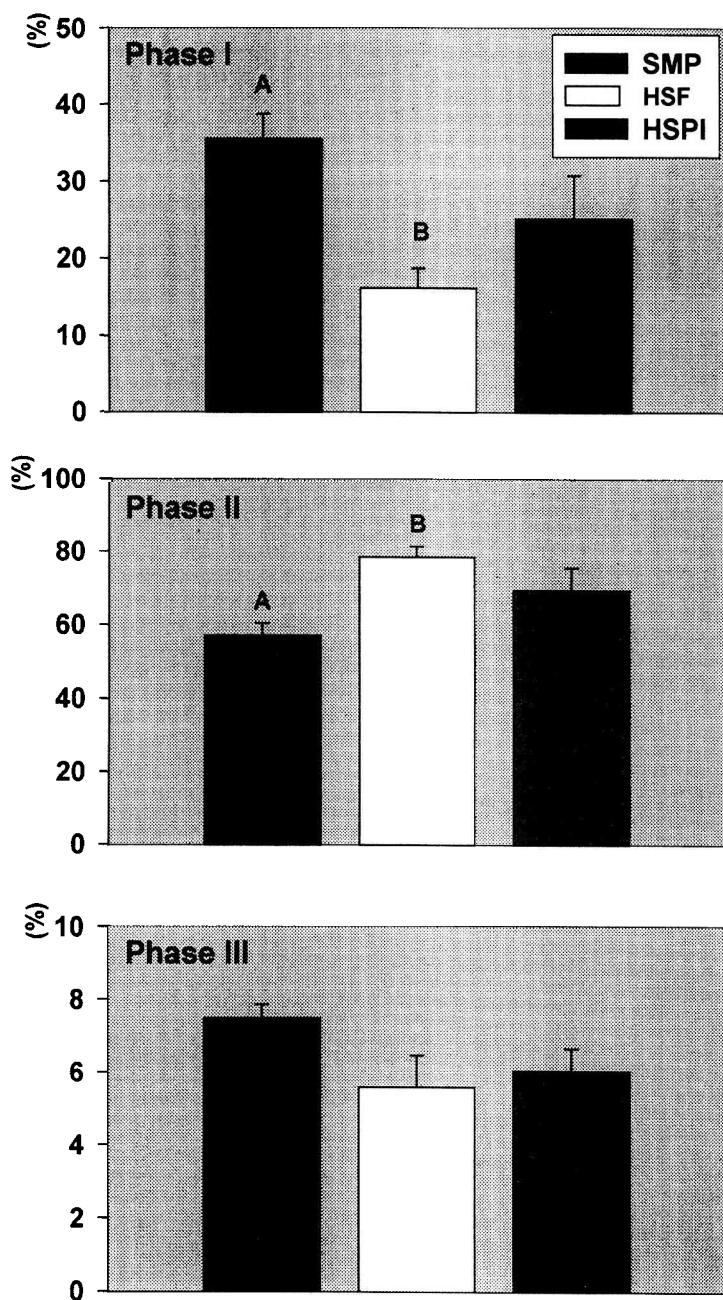
\*, \*\* Overall effect of treatment (\*  $P < 0.05$ , \*\*  $P < 0.01$ ).

A,B,a,b Means with different superscripts in the same column are significantly different (lower case letters  $P < 0.05$ , upper case letters  $P < 0.01$ ).

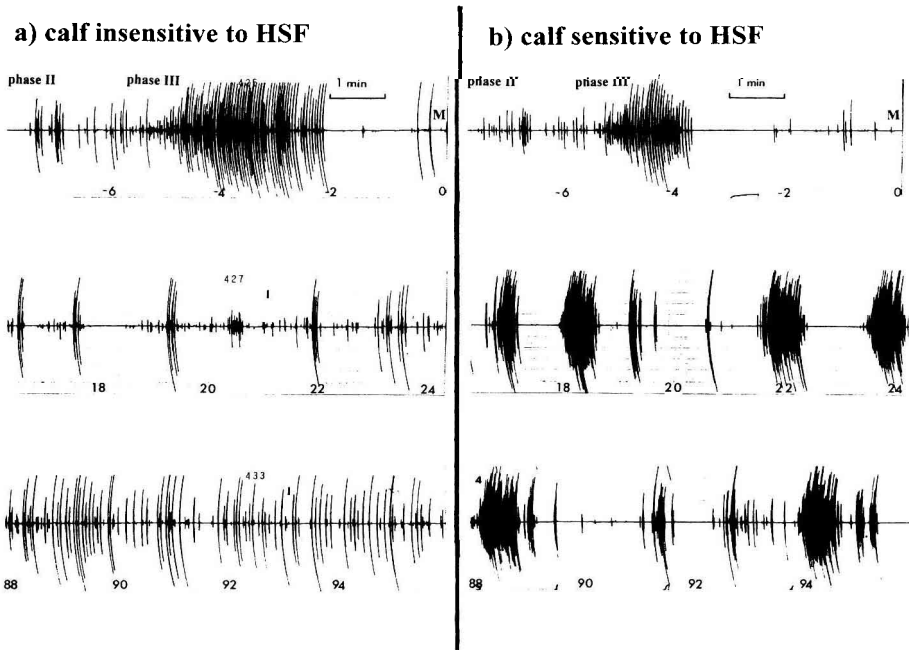
MMC, myoelectric migrating complex.



**Figure 1.** Jejunal myoelectric activity integrated over 7 h after the morning meal in a soya-sensitive pre-ruminant calf given test meals containing either skim milk powder (SMP), antigenic heated soyabean flour (HSF) or non-antigenic hydrolysed soya protein isolate (HSPI). A myoelectric migrating complex comprises phase I (quiescence), phase II (irregular spiking activity) and phase III (regular spiking activity).



**Figure 2.** Total durations (%) of phases I, II and III of myoelectric activities recorded on the jejunum over 7 h in four soya-sensitive preruminant calves given test meals containing either skim milk powder (SMP), antigenic heated soyabean flour (HSF) or non-antigenic hydrolysed soya protein isolate (HSPI). Means and SEM. <sup>A,B</sup> Bars with different letters differ significantly ( $P < 0.01$ ).



**Figure 3a, 3b.** Instantaneous myoelectric activities recorded before and after a test meal containing antigenic heated soyabean flour (HSF) on the jejunum of (a) a calf insensitive to HSF and (b) a calf sensitive to HSF. Note in *figure 3b* (calf sensitive to HSF) the bursts of myoelectric activities appearing 17 min after the meal and which are still present after 95 min. (M = meal given).

phases I, II and III were intermediate between their respective counterparts determined with test meals containing SMP or HSF (*table I*). Differences in the mean duration of phase III between SMP and HSPI remained significant ( $P < 0.05$ ). Phases I, II and III represented 25.1, 69.1 and 6.0 % of the recording time with HSPI (*figure 2*). Diarrhoea was observed on one of eight occasions only.

### 3.2. Influence of pharmacological pretreatment

The calves sensitive to HSF were given test meals based on HSF and received either no medication or a drug 30–60 min

prior to feeding. One calf died for unknown reasons before the completion of this part of the study, leading to only three full sets of data for statistical analysis. The characteristics of the myoelectric activity with HSF alone was close to those described above (*table II*). The number of phases I over 7 h was significantly lower with promethazine than indomethacin pretreatment ( $P < 0.01$ ). Significant overall effects of treatments were also detected for the mean durations of phases I, II and III ( $P < 0.05$  to  $P < 0.01$ ). In particular, there was a tendency for the mean duration of phase I to increase (+40 %) with promethazine and to decrease (–37 %) with indomethacin premedications, as compared with HSF alone ( $P = 0.07$

**Table II.** Influence of promethazine or indomethacin premedication on the characteristics of jejunal myoelectric activity following a test meal based on heated soyabean flour (HSF) in three calves previously fed antigenic soya for 3 months and receiving each test meal on two different occasions (means  $\pm$  SEM).

Treatment	Frequency of MMC (no./h)	Mean MMC duration (min)	Number of phases I (no./7 h)	Mean duration (min) of:			Frequency of diarrhoea
				Phase I**	Phase II*	Phase III**	
HSF alone	0.90 $\pm$ 0.15	81.9 $\pm$ 14.4	4.2 <sup>AB</sup> $\pm$ 0.5	13.9 $\pm$ 2.3	66.4 $\pm$ 12.7	3.94 $\pm$ 0.33	3 / 6 (2 calves)
Promethazine/ HSF	0.73 $\pm$ 0.05	84.2 $\pm$ 5.7	3.0 <sup>A</sup> $\pm$ 0.4	19.5 $\pm$ 3.8	64.8 $\pm$ 6.8	4.47 $\pm$ 0.51	1 / 6
Indomethacin/ HSF	0.98 $\pm$ 0.09	63.3 $\pm$ 5.9	5.2 <sup>B</sup> $\pm$ 0.5	8.7 <sup>#</sup> $\pm$ 2.0	50.1 $\pm$ 3.2	3.87 $\pm$ 0.19	3 / 6 (3 calves)

\*, \*\* Overall effect of treatment (\*  $P < 0.05$ , \*\*  $P < 0.01$ ).

<sup>A,B</sup> Means with different superscripts in the same column are significantly different ( $P < 0.01$ ).

#  $P = 0.07$  between promethazine and indomethacin premedications.

MMC, myoelectric migrating complex.

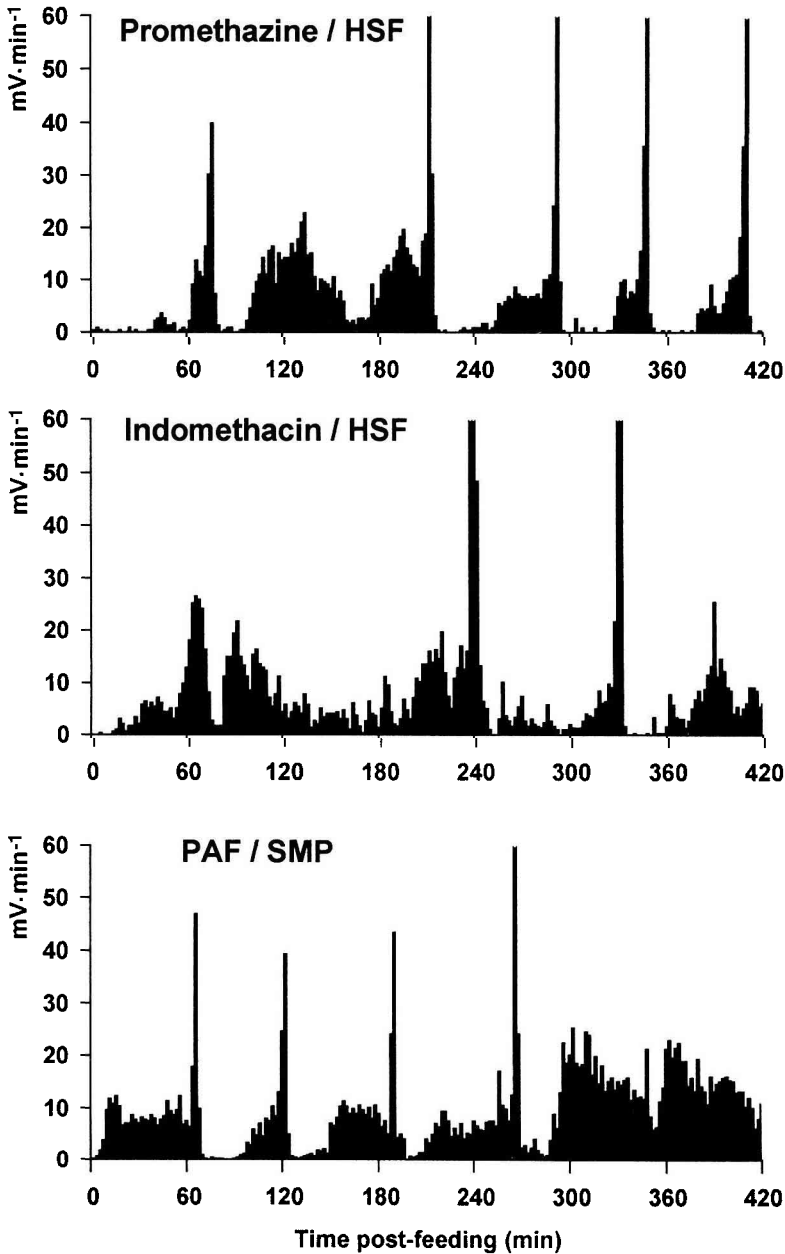
between promethazine and indomethacin). Also, diarrhoea was observed on only one of six occasions with promethazine + HSF as compared with three occasions (three calves) with HSF alone or indomethacin + HSF (table II). Typical integrated myoelectric activity recordings observed with promethazine or indomethacin premedication are provided in figure 4.

The administration of PAF prior to a meal based on SMP reduced significantly the mean duration of phase III ( $P < 0.02$ ) (table III). The total duration of phase III over the 7 h period of recording also tended to be lower with PAF pretreatment than with SMP alone [ $5.85 \pm$  (SEM)  $0.52$  % versus  $7.59 \pm 0.46$  %,  $P = 0.059$ ]. Finally, PAF administration induced diarrhoea on two of six occasions. A typical integrated myoelectric activity recording observed with PAF pretreatment is shown in figure 4.

#### 4. DISCUSSION

Alteration of the myoelectric activity pattern along the gut is a general response

to diarrhoeal diseases of various causes including food, infection and parasitism (reviews by Crowe and Perdue [4]; Navarre and Roussel [20]). It constitutes a mechanism of defence to clear noxious substances from the gut lumen. In our study, the main motor changes induced by the consumption of antigenic soya and associated diarrhoea were reduced durations of phases I and III, i.e. quiescence and regular spiking activity, respectively. Conversely, the total duration of phase II, i.e. irregular spiking activities, increased. This is in general agreement with observations related to enterotoxin-induced diarrhoea in calves [24]. Here, the total duration of phases I and III decreased from 52 to 27 % and from 12 to 6 %, respectively, while that of phase II increased from 37 to 67 % of the recording time, following oral administration of 200  $\mu$ g of heat-stable enterotoxin from *Escherichia coli*. Roussel et al. [24] noted the occurrence of numerous clusters of minute rhythms. They suggested that these are central to the onset of diarrhoea, probably in response to excessive luminal distension, irrespective of the causative agent. In the



**Figure 4.** Jejunal myoelectric activity integrated over 7 h after the morning meal in the same soya-sensitive preruminant calf as in *figure 1*. This calf had been pretreated with promethazine or indomethacin prior to being given a test meal containing antigenic heated soybean flour (HSF), or with PAF prior to being given a test meal containing skim milk powder (SMP). Note the differences with motor patterns for a calf showing sensitivity to HSF in *figure 1*.

**Table III.** Influence of platelet-activating factor pretreatment on the characteristics of jejunal myoelectric activity following a test meal based on skim milk powder in three calves previously fed antigenic soya for 3 months and receiving each test meal on two different occasions (means  $\pm$  SEM).

Treatment	Frequency of MMC (no./h)	Mean MMC duration (min)	Mean duration (min) of:			Frequency of diarrhoea
			Phase I**	Phase II*	Phase III**	
SMP alone	0.85 $\pm$ 0.04	71.5 $\pm$ 3.8	29.6 $\pm$ 3.5	38.0 $\pm$ 2.0	5.67 <sup>a</sup> $\pm$ 0.26	0 / 6
Platelet-activating factor / SMP	0.89 $\pm$ 0.03	67.8 $\pm$ 2.2	23.0 $\pm$ 5.1	36.7 $\pm$ 3.2	4.59 <sup>b</sup> $\pm$ 0.18	2 / 6 (2 calves)

<sup>a, b</sup> Means with different superscripts in the same column are significantly different ( $P < 0.02$ ). MMC: myoelectric migrating complex.

present experiment, minute rhythms were observed occasionally while diarrhoea was frequent with HSF. Therefore, it seems from our observations that decreases in mean and/or total durations of phases I and III were more consistent criteria associated with diarrhoea.

Contrasting with our results were those of Sissons et al. [32, 33] and Duvaux [8], whose studies indicated that the disturbed motility pattern after HSF challenge comprised a 2-fold increase in the number of MMCs, a reduced phase II component and an enhanced number of phase III activities. Such variations may have been related to differences in the level of milk intake and/or way of sensitization between the studies. First, increasing milk intake in healthy calves was shown to induce a linear decrease in the number of MMCs, a quadratic shortening of phase I and a linear increase in the mean duration of phase II activities [12]. The level of intake was 3- to 4-fold higher in the present study compared with those in experiments by Sissons and Duvaux. In a rat model of food-induced anaphylaxis, differences were also noted in myoelectric disorders between the fasted and fed states. Antigen challenge carried out during fasting completely abolished the MMC patterns which were

replaced by a long-lasting phase II [27]. When the challenge occurred after a meal, the post-prandial phase II, characteristic of the fed state, was maintained, but there was an increase in the number of high-amplitude clustered contractions [7]. Thus, the state of repletion of the gut affects different aspects of the myoelectric activity along the small intestine in both physiological and hypersensitive situations.

Another difference between the experiments with calves is related to the mode of sensitization to soya. In the present studies, the calves were fed continuously the milk replacer containing HSF, as normal husbandry practice. By contrast, Sissons et al. [32, 33] and Duvaux [8] fed test meals of HSF to their calves twice a week. This may have had consequences on the type of hypersensitivity reactions that developed. For example, data gathered by Sissons and co-workers were more indicative of a type I, IgE-mediated gut hypersensitivity, as suggested by PCA-positive sera and immediate direct skin reactions [13, 15]. It is also well known that low doses of antigen stimulate, while repeated antigen exposure attenuates, the IgE response (review by Crowe and Perdue [4]). In contrast, our calves did not show detectable amounts of specific IgE

antibodies either in plasma or in intestinal secretions (Dréau and Lallès, unpublished data) as assessed using anti-bovine IgE monoclonal antibodies [35]. Our calves, however, displayed long-lasting skin reactions and specific lymphoproliferation *in vitro*, both being more suggestive of type IV, T-cell mediated, hypersensitivity [17]. To our knowledge, there is no direct evidence to support a link between these types of hypersensitivity on subsequent motility disturbances. However, the repeated antigen challenge of rats previously sensitized by intraperitoneal immunization affects various aspects of mast cell populations and functions [5, 19, 39]. This in turn may modify some parameters of motility disorders described in this model (see below).

The biochemical mediators involved in motility disturbances associated with diarrhoea may depend on different factors, including: the causative agent, the animal species, the site of observation along the gut and the type of experimental approach used (*in vivo* or *in vitro*). In a rat model of food-induced intestinal anaphylaxis, *in vivo* and *in vitro* data both indicate that 5-hydroxytryptamine (via 5-HT-1, 5-HT-2 and probably 5-HT-3 receptors) and arachidonic acid derivatives of the cyclooxygenase and lipoxygenase pathways (i.e. prostaglandins and leukotrienes, respectively) are major mediators acting on longitudinal and circular smooth muscles of the small intestine, while histamine plays a minor role [4, 10, 28]. In contrast, 5-hydroxytryptamine did not seem to be implicated in the antigen-induced contraction of colonic segments *in vitro* while leukotriene D<sub>4</sub> and PAF did appear to play a role [21]. The biphasic contraction observed *in vivo* with segments of colon from guinea pigs orally sensitized to  $\beta$ -lactoglobulin showed histamine involvement in the early stage and leukotrienes in the late stage [1].

Most of the mediators are released from mucosal mast cells upon specific IgE-mediated antigen stimulation (review by Crowe and Perdue [4]; Fargeas et al. [10]). This release also depends on neural circuits, including at least one nicotinic synapse in the ganglionic pathway and muscarinic cholinergic excitation of intestinal smooth muscle, substance P and capsaicin-sensitive afferent nerves [11, 29]. The central nervous system is also implicated through *c-fos* expression [2]. This implies that vagal afferents at least monitor the intestinal response or might be the afferent sensory limb of an intestinal motor response programmed centrally [29]. Finally, intestinal motor disorders induced by *E. coli* enterotoxin in the rat involve PAF whose action is partly mediated by prostaglandins and free radicals [23].

The situation is far less clear in the calf. Our observations suggest the involvement of histamine via H-1 receptors while arachidonic catabolites of the cyclooxygenase pathway probably play a minor role. This is consistent with the inhibition of skin responses to soya by the H-1 receptor antagonist promethazine with these calves [17]. However, Pedersen [22] failed to detect increased concentrations of 3-methyl-histamine in the urine of calves sensitive to soya. Information on function and tissue distribution of H-1 receptors in ruminants is scarce. Ruckebusch and Soldani [25] showed the excitatory role of histamine in the ovine proximal duodenum, an effect selectively and totally blocked by promethazine. Conversely, H-2 receptors were inhibitory. The gene coding for bovine H-1 receptor has been recently cloned [41]. It encodes a protein of 491 amino acids (56 kDa) and the corresponding mRNA is expressed to a greater extent in the small intestine and lung than in the adrenal medulla and uterus. The origin of histamine is still uncertain but it could come from mast

cells which are more numerous in intestinal tissues of sensitive calves [32]. The influence of PAF should have been tested using appropriate PAF receptor antagonists in this study. However, the observation that PAF administration disturbed the motility patterns in a way comparable to HSF may indirectly suggest PAF implication. Indeed in the calf, sucrose induces diarrhoea and motor disturbances whose patterns are quite different from those induced by HSF [8, 9]. Besides, Denizot et al. [6] demonstrated substantial amounts of PAF in the watery stools of patients suffering from Crohn's disease (i.e. inflammatory state) but not when diarrhoea resulted from malabsorption (i.e. non-inflammatory state). Therefore, our results with PAF indirectly suggest an inflammatory state of the small intestine in the present study.

The role of a larger array of cells and mediators is worthy of consideration because many are known to interfere with intestinal epithelial function, i.e. absorption, secretion and permeability (review by Theodorou et al. [37]). However, little is known of their impact on motility. T lymphocytes have been shown to modulate intestinal muscle function in *Trichinella*-infected rats [40] but IL-1, IL-6 and TNF- $\alpha$  did not seem to contribute to the increased contraction of longitudinal muscle in vitro in this model [14]. In contrast, IL-1 is involved in colonic motor disorders and secretory changes induced by food anaphylaxis in the guinea pig [36]. We demonstrated an increase in the density of T cells in the intestinal tissues of calves fed chronically antigenic soya [18]. Whether this is relevant to the alterations of motility patterns reported here remains to be established.

In conclusion, calves chronically fed antigenic soya become sensitized and develop intestinal motility disorders specifically towards dietary antigens. This appears to involve histamine through H-1

receptors, possibly PAF, but probably not cyclooxygenase catabolites of arachidonic acid. Further work is needed to study the implication of 5-hydroxytryptamine and other mediators such as interleukins, as well as that of cells located in the gut.

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