

Identification of soyabean allergens and immune mechanisms of dietary sensitivities in preruminant calves

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SUMMARY

The allergenicity of soya proteins was assessed by direct skin testing and by *in vitro* lymphoproliferation tests in calves fed milk substitutes containing skim milk powder (SMP) or an antigenic heated soya flour (HSF). During the last three weeks of treatment, the calves were injected intradermally with raw soya flour (RSF), HSF, hydrolysed soya protein isolate (HSPI), SMP or purified soya proteins, after being premedicated with anti-histamine or not. Peripheral blood leucocytes (PBL) were grown over five days with various mitogens or dietary antigens, and the incorporation of tritiated thymidine was measured. Strong skin oedema reactions to RSF, HSF and all the purified proteins were observed in the calves fed HSF at various times up to 24 hours after injection. The skin oedema was largely prevented by premedication with anti-histamine. A strong delayed skin thickening was observed in the calves fed HSF for up to five days with β -conglycinin. PBL from the calves fed HSF proliferated *in vitro* with HSF, HSPI and β -conglycinin, but not with glycinin. Thus, most proteins from soyabean were implicated in the immediate and semi-delayed immune reactions, whereas β -conglycinin was strongly involved in a delayed type hypersensitivity in calves.

PRERUMINANT calves fed insufficiently processed soya products develop immune-mediated gut disturbances leading to malabsorption, poor growth and, in extreme circumstances, to death (review by Sissons 1993). Earlier work indicated that both immediate (type I), that is IgE mediated (Kilshaw and Sissons 1979a) and semi-delayed (type III), that is immune-complex mediated (Barratt et al 1979), reactions might be implicated in these functional and morphological intestinal disorders.

Direct skin testing with saline extracts of heated soya flour (HSF) confirmed the former mechanism (Heppell et al 1987). No convincing evidence, that is the presence and the deposition of specific immune complexes in gut tissues, has been published with respect to type III reactions.

Attempts to demonstrate the involvement of delayed (type IV), that is cell-mediated, hypersensitivity remained unsuccessful as judged by the lack of skin reactions 24 hours after the intradermal injection of HSF extracts (Heppell et al 1987) and the absence of specific *in vitro* proliferation of peripheral blood leucocytes (PBL) in calves fed HSF (Dawson et al 1988).

Finally, allergens from soya have never been identified in calves, although agglutinating antibodies against the major storage globulins glycinin and β -conglycinin have been demonstrated in the blood (Kilshaw and Sissons 1979b). An experiment was therefore designed to study the effects of purified soya proteins on both skin tests and *in vitro* lymphoproliferation, in calves fed control or HSF milk replacer diets.

The results indicate that most of the proteinaceous constituents of soya, including the storage globulins, protease inhibitors and lectins, are involved in short-term histamine-dependent skin reactions. β -conglycinin induced both strong delayed skin thickening and *in vitro* lymphoproliferation of PBL.

MATERIALS AND METHODS

Animals and diets

Thirty-two male Holstein calves were obtained at two days of age from the experimental dairy herd at INRA, Rennes. They were kept on straw in individual pens (1.5 x 2.5 m) throughout the experiment. They were fed a commercial milk replacer diet containing skim milk powder (SMP) as the only source of protein up to three to four weeks of age. The 24 control animals continued to consume this diet up to the end of the trial. The remaining eight calves were fed for three months on a milk substitute diet whose protein was provided by a mixture (70:30 on a crude protein [CP] basis) of heated soyabean flour (HSF, Société Industrielle des Oléagineux, Bougival, France) and whey. Details of the diets have been published by Lallès et al (1995). The HSF contained 3.9 and 3.6 per cent (CP basis) of immunoreactive glycinin and β -conglycinin respectively, as determined by ELISA (Tukur et al 1993). All the calves were fed the diets, at 50 g dry matter kg^{-1} metabolic weight day^{-1} , in open pails twice daily at 08.00 and 16.00 hours. Bodyweight was recorded weekly.

Specific antibody production

The humoral response of the calves to HSF was determined by the passive haemagglutination test (Kilshaw and Sissons 1979a), using a saline extract of HSF as the coating antigen mixture (1 mg ml^{-1}). The results were expressed as titres, that is the number of doubling dilutions of sera from an initial dilution of 1:20 above which the haemagglutination disappeared (Kilshaw and Sissons 1979a).

Test substances

Glycinin, α -conglycinin and β -conglycinin were purified as described by Thanh and Shibasaki (1976), Koshiyama et

al (1981) and King (1977), respectively. The proteins were then dialysed against distilled water, freeze-dried and resuspended (5 mg ml^{-1}) in phosphate buffered saline (PBS, pH 7.4) prior to the injections. Soyabean Bowman-Birk protease inhibitors (BBI) and lectins were purchased from Sigma (St-Louis, MO, USA). Protein extracts (5 mg ml^{-1}) were prepared in PBS from raw soya flour (RSF), HSF, hydrolysed soya protein isolate (HSPI, Lallès et al 1995), SMP, and bovine serum albumin (BSA grade IV, Sigma). An aqueous solution of histamine dihydrochloride (10^{-3}M , Sigma) was also prepared.

Skin tests

Both flanks of the calves were shaved two days before the skin tests. Twelve squares of side 8 cm were drawn on each flank. A tranquiliser (Ventranquil, Sanofi, Libourne, France) was administered intravenously (0.03 ml kg^{-1}) one hour before the skin testing. The test substances were injected intradermally (0.2 ml , that is, 1 mg for the protein solutions) at random into both flanks. The skin oedema diameter (SOD) and double skin fold thickness (DSFT) were measured over 24 hours and five days respectively, as described by Heppell et al (1987). The calves were randomly skin tested twice after nine and 12 weeks of treatment, being either premedicated with an H-1 histamine receptor antagonist (promethazine, 2 mg kg^{-1} intravenously) (Rhône-Mérieux, Lyon, France) or not. Two different groups of eight control calves were used each time to avoid unwanted responses due to a possible sensitisation to the soya compounds injected into the skin.

Lymphoproliferation in vitro

The calves fed HSF were maintained on the control milk substitute for two weeks before samples of jugular blood were collected. Eight naive calves fed SMP were used as controls. Peripheral blood leucocytes (PBL) were prepared by the method described by Hammerberg et al (1989). Cell-mediated immune responses were measured by means of a blastogenic assay described by Bendich et al (1984). PBL were diluted ($10^6 \text{ cells ml}^{-1}$) in RPMI 1640 (Gibco, Gaithersburg, Maryland, USA) supplemented with 10 per cent fetal calf serum, 0.2 mM glutamine (Gibco), 0.25 mM HEPES (Sigma), 0.1 mM pyruvate (Gibco), 2 µg ml^{-1} fungizone (Gibco), 80 µg ml^{-1} gentamycin (Sigma) and 50 µM β -mercapto-ethanol (Sigma). The PBL were distributed in triplicate into wells of microtitre plates (Falcon 3072, Becton-Dickinson, Lincoln, New Jersey, USA). They were incubated with various mitogens and specific dietary antigens diluted in the culture medium (Table 4). After 96 hours at 37°C in a 5 per cent carbon dioxide environment, PBL proliferation was measured as tritiated thymidine uptake. The cells were pulsed with 1 µCi per well of ^3H -thymidine (NEM, Du Pont Company, Wilmington, Denver, USA) during the last 18 hours of culture. They were harvested on filter papers and radioactivity was counted in a scintillation cocktail (Packard Company, Meriden, Connecticut, USA). The results were expressed as counts per minute (cpm, Table 4).

Statistical analysis

Data from the skin and in vitro lymphoproliferation tests, carried out in duplicate and triplicate respectively, were averaged before statistical analysis. The differences between animal groups were analysed by the non-paramet-

ric test of Mann-Whitney and the kinetic data were analysed by that of Friedman (Hollander and Wolfe 1973). The effect of the injection of soya protein as compared to the injection of SMP within a group of calves was analysed by the Wilcoxon test (Hollander and Wolf, 1973). Pooled SE or SEM are provided for descriptive information only and are not part of the non-parametric tests.

RESULTS

Animal performance and anti-soya antibodies

The calves fed the control diet remained healthy and grew satisfactorily throughout the trial (with a mean [SEM] bodyweight gain of $1.21 [0.02] \text{ kg day}^{-1}$) (Fig 1). In contrast, the calves fed the milk substitute containing HSF grew more slowly ($0.64 [0.05] \text{ kg day}^{-1}$, $P < 0.01$) (Fig 1). This difference was due partly to the lower feed intake of the calves fed HSF, and partly to the lower apparent digestibility of the protein from HSF (Lallès et al 1995). Diarrhoea was occasionally observed in the calves fed HSF. Anti-soya antibody titres in the sera were below unity for both groups of animals before the start of the experiment; the titres remained low in the control group but increased markedly ($P < 0.01$) in the HSF group (Fig 2).

Direct skin tests

Changes in mean SOD up to 24 hours after the intradermal injection are shown in Table 1. As expected, neither saline nor BSA induced significant skin reactions. A mean (SEM) SOD reaction of $29.4 (3.96)$ and $24.8 (1.76) \text{ mm}$ was observed one hour after the histamine injection in the control calves and the calves fed HSF, respectively; the difference between the groups was not significant ($P > 0.05$). The injection of SMP and unpurified soya protein (RSF, HSF and HSPI) provoked only slight oedema reactions ($< 5 \text{ mm}$) in the control calves one hour after injection (significant with

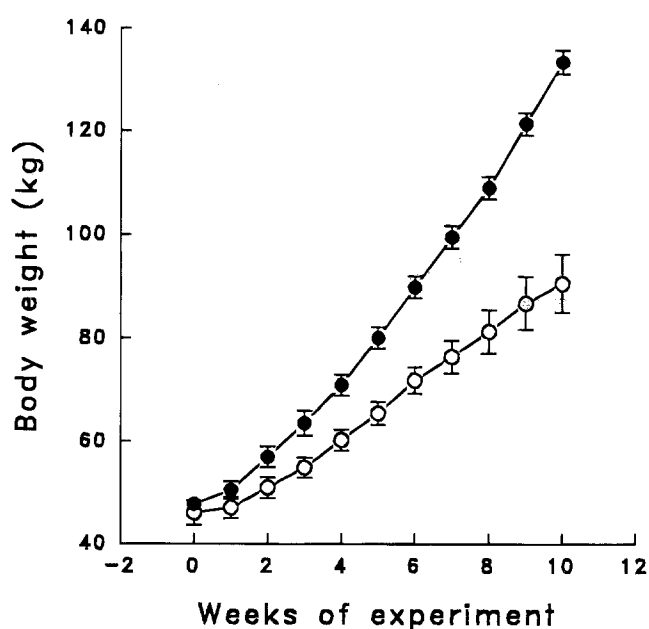


FIG 1: Mean (SEM) changes in the bodyweight of calves fed milk replacers containing skim milk powder (SMP) (●) or heated soya flour (HSF) (○) as the main protein source. There were significant differences between the treatments after two and three weeks ($P < 0.05$), and from four weeks onwards ($P < 0.01$).

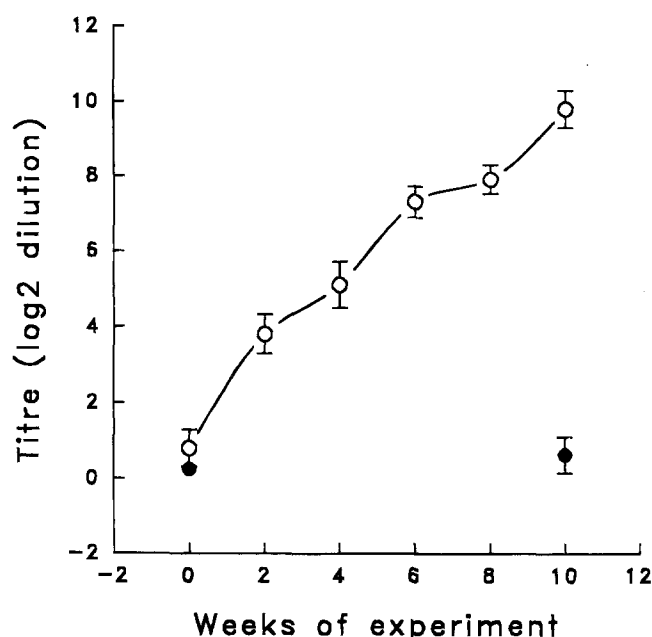


FIG 2: Mean (SEM) changes in the anti-soya antibody titre in the plasma of calves fed milk replacers containing skim milk powder (SMP) (●) or heated soya flour (HSF) (○) as the main protein source. There was a significant difference between the treatments at week 10 ($P<0.01$) and a significant effect of time in the calves fed HSF ($P<0.01$).

HSF and HSPI, $P<0.05$). In contrast, the calves fed HSF displayed significantly larger reactions (11 to 17 mm, $P<0.01$) to RSF and HSF than the controls, one to eight hours after injection. The calves fed HSF also showed a slight but significant ($P<0.05$) oedema with SMP. The differences between the groups were not significant for HSPI, regardless of time. Skin oedema due to the purified proteins was particularly severe (up to 18 mm) and persistent (up to eight hours) with α -conglycinin, β -conglycinin and lectins in the control calves (Table 1); it was moderate with BBI and absent with glycinin. All the five purified proteins studied induced specific oedema responses (up to 30 mm, $P<0.05$ to $P<0.01$) in the calves fed HSF when compared with the controls (Table 1). However, the kinetics of the reaction differed among the proteins because the maximal net oedema, that is, the values in the HSF group minus the values in the control group, was observed after four hours for glycinin (mean diameter 10 mm), after eight hours for α -conglycinin, BBI and the lectins (15 mm), and after one and eight hours for β -conglycinin (11 and 17 mm, respectively). It is also noteworthy that β -conglycinin was responsible for the largest and most persistent skin reaction.

TABLE 2: Influence of anti-histamine premedication on the mean diameter of skin reaction (mm) to the intradermal injection of various solutions into calves fed milk replacers containing skim milk powder (SMP) or heated soya flour (HSF) as the main protein source for three months

Solution injected	Calves fed SMP		Calves fed HSF		Pooled SE
	1 hour	8 hours	1 hour	8 hours	
SMP	0.6	0.0	1.3	0.4	0.27
RSF	0.4	0.0	7.2**	1.8	0.69
HSF	3.5	0.0	6.1	2.3	0.72
HSPI	1.5	0.0	0.0	0.0	0.26
Glycinin	0.4	0.0	5.3*	1.4	0.65
α -conglycinin	3.8	1.6	9.4	4.3	0.82
β -conglycinin	4.1	2.1	11.5*	5.8	1.06
BBI	1.6	0.7	1.2	0.6	0.41
Lectins	3.9	2.6	0.7	6.1	0.73

* $P<0.05$, ** $P<0.01$ (Mann-Whitney test) between SMP and HSF-fed calves, for a given time after injection in the same row

RSF Raw soya flour, HSPI Hydrolysed soya protein isolate, BBI Bowman-Birk protease inhibitors

The influence of premedication with a histamine-receptor antagonist on the skin oedema reaction is summarised in Table 2. The mean SOD was smaller than the values in Table 1, and most of the differences between the groups of calves had disappeared.

The changes in mean DSFT up to five days after the intradermal injections are shown in Table 3. Skin thickening was usually small (<0.5 mm) in both experimental groups for the control substances, that is, saline, BSA, histamine and SMP. In the control group, β -conglycinin, and to a smaller extent α -conglycinin, BBI and the lectins, induced significantly stronger reactions ($P<0.05$ to $P<0.01$) than the injected SMP. Extracts from RSF and HSF induced a significantly ($P<0.05$ to $P<0.01$) larger increase in DSFT at one and four days after injection in the calves fed HSF than in the controls. This appeared to be due mainly to the specific effects of β -conglycinin (up to 3.7 mm of increase in DSFT, $P<0.01$) between days 1 and 5. The contribution of α -conglycinin to this reaction (1.1 mm at day 1), although significant ($P<0.05$), was more limited. In contrast, the differences between calf groups for changes in DSFT were extremely small and not significant as far as glycinin, BBI and the lectins were concerned.

Lymphoproliferation tests in vitro

The incorporation of ^3H -thymidine by PBL grown over five days in vitro under various conditions is given in Table 4. The spontaneous cell division as well as the mitogenesis induced by pokeweed mitogen (PWM) lipopolysaccharide (LPS) and concanavalin A (Con A) were not statistically

TABLE 1: Changes with time in the mean diameter (mm) of the skin reaction to the intradermal injection of various solutions in calves fed milk replacers containing skim milk powder (SMP) or heated soya flour (HSF) as the main protein source for three months

Solution injected	Calves fed SMP				Calves fed HSF				Pooled SE
	1 hour	4 hours	8 hours	24 hours	1 hour	4 hours	8 hours	24 hours	
SMP	1.4	3.6	1.2	0.2	5.1*	5.0	4.7	0.0	0.46
RSF	2.9	1.9	0.0	0.0	12.6**	17.0**	11.3*	5.0	1.01
HSF	4.8 ^a	1.9	0.3	0.0	13.4**	15.9**	10.7**	3.7	0.94
HSPI	4.7 ^a	0.5	0.0	0.0	5.2	0.6 ^a	0.6	0.0	0.37
Glycinin	1.3	1.0	1.3	0.0	7.6*	10.8*	5.1	0.0	0.72
α -conglycinin	10.4 ^A	14.5 ^A	7.9 ^A	1.9	14.3 ^A	23.9 ^{A*}	22.0 ^{A*}	8.1 ^{A*}	1.18
β -conglycinin	11.7 ^A	15.2 ^A	10.3 ^A	6.4 ^a	22.5 ^{A**}	22.6 ^{A*}	26.8 ^{A**}	19.1 ^{A**}	1.10
BBI	5.9 ^A	9.8 ^A	2.4	0.0	5.1	16.6 ^A	17.4 ^{a**}	3.3	1.07
Lectins	6.9 ^a	18.2 ^A	14.2 ^A	5.6 ^a	9.1	20.7 ^A	28.3 ^{A*}	14.8 ^{A*}	1.27

^a $P<0.05$, ^A $P<0.01$ (Wilcoxon test) from SMP injected, for a given time after injection in the same column

* $P<0.05$, ** $P<0.01$ (Mann-Whitney test) between SMP and HSF-fed calves, for a given time after injection in the same row

RSF Raw soya flour, HSPI Hydrolysed soya protein isolate, BBI Bowman-Birk protease inhibitors

TABLE 3: Changes with time in the mean double skin fold thickness (mm) after the intradermal injection of various solutions in calves fed milk replacers containing skim milk powder (SMP) or heated soya flour (HSF) as the main protein source for three months

Solution injected	Calves fed SMP					Calves fed HSF					Pooled SE
	1 day	2 days	3 days	4 days	5 days	1 day	2 days	3 days	4 days	5 days	
SMP	0.06	0.12	0.13	0.17	0.45	0.16	0.23	0.31	0.33	0.33	0.045
RSF	0.13	0.23	0.16	0.00	0.46	0.89 ^A *	0.33	0.26	0.31	0.38	0.049
HSF	-0.10	-0.10	0.00	-0.20	0.24	1.07 ^A **	0.38*	0.26	0.47*	0.35	0.058
HSPI	0.15	0.13	0.16	0.17	0.53	0.00	0.08	0.22	0.24	0.24	0.039
Glycinin	0.31	0.15	0.17	0.11	0.51	0.34 ^a	0.23	0.17	0.21	0.26	0.046
α -conglycinin	0.40 ^a	0.25	0.14	-0.10	0.35	1.53 ^A *	0.74	0.57	0.56	0.44	0.078
β -conglycinin	1.19 ^A	0.93 ^A	0.69 ^A	0.35	0.82	4.85 ^A **	4.41 ^A **	3.77 ^A **	3.68 ^A **	3.30 ^A **	0.218
BBi	0.42 ^a	0.19	0.11	0.06	0.37	0.84 ^A	0.54	0.36	0.31	0.44	0.057
Lectins	1.69 ^A	0.71	0.47	0.16	0.53	2.66 ^A	1.23 ^A	0.79	0.73 ^a	0.56	0.112

^a $P < 0.05$, ^A $P < 0.01$ (Wilcoxon test) from SMP injected, for a given time after injection in the same column

* $P < 0.05$, ** $P < 0.01$ (Mann-Whitney test) between SMP and HSF-fed calves, for a given time after injection in the same row

RSF Raw soya flour, HSPI Hydrolysed soya protein isolate, BBi Bowman-Birk protease inhibitors

TABLE 4: Mean (SEM) in vitro lymphoproliferation (cpm $\times 10^{-3}$) of peripheral blood lymphocytes (PBL) collected from groups of six calves fed milk replacers containing either skim milk powder (SMP) or heated soya flour (HSF) as the main protein source for three months

Mitogen or antigen	Concentration ($\mu\text{g ml}^{-1}$)	Calves fed SMP		Calves fed HSF		P*
		Mean	SEM	Mean	SEM	
RPMI 1640	0	1.81	0.60	4.0	1.92	NS
PWM	10	153	29.4	148	23.6	NS
LPS	20	10.8	3.97	10.4	3.93	NS
Con A	50	141	41.3	152	25.5	NS
HSF	100	1.55	0.43	8.84	2.37	0.02
	250	2.49	0.71	11.3	3.38	0.03
HSPI	100	2.05	0.89	9.16	4.04	0.05
	250	2.06	0.68	12.5	5.15	0.05
Glycinin	100	1.61	0.48	3.68	1.18	NS
	250	0.65	0.16	0.89	0.28	NS
β -conglycinin	100	1.37	0.22	7.21	2.32	0.02
	250	0.70	0.07	2.00	0.79	NS

* Difference between calf groups (Mann-Whitney test). NS Not significant
PWM Pokeweed mitogen, LPS Lipopolysaccharide, Con A Concanavalin A, HSPI Hydrolysed soya protein isolate

different between the groups of calves. In contrast, extracts of HSF, HSPI and β -conglycinin, but not glycinin, were responsible for a significantly ($P < 0.05$) higher ^3H -thymidine incorporation in PBL collected from the calves fed HSF than from the controls. The mean individual stimulation indices (the cpm in stimulated culture divided by the cpm in the unstimulated culture) ranged between 4 and 8 for HSF and HSPI, and between 1 and 4 for β -conglycinin, depending on the antigen concentration.

DISCUSSION

Soyabean is a major source of protein for animal feeding because of its high nutritional and economical value. The storage globulins glycinin (11S fraction) and β -conglycinin (7S) represent approximately 40 and 20 per cent of the protein in the bean (Guéguen and Azanza 1985). A small 2S fraction (α -conglycinin) found with the 11S and 7S fractions shares common properties with the Kunitz inhibitors (Koshiyama et al 1981). Protease inhibitors of the Kunitz and Bowman-Birk families and lectins are mainly found in the whey fraction (Guéguen and Azanza 1985). Glycinin and β -conglycinin are resistant to digestion in the calf (Sissons 1993, Tukur et al 1993). Protease inhibitors and lectins are well known for their anti-nutritional properties. The trypsin inhibitors of the Kunitz family reduce the digestion and absorption of dietary proteins and drastically

increase the loss of endogenous proteins in the gut of pigs (Barth et al 1993). In rats, soyabean lectins resist digestion, bind to the intestinal epithelium, induce hyperplasia of the enterocytes and elongation of the crypts leading to impaired growth (Pusztai et al 1990). These adverse effects have not been demonstrated in the calf, although it is known that soyabean lectins bind to the epithelium of its gut (Hendriks et al 1989).

The present data indicate that glycinin and β -conglycinin may be implicated in immediate, type I, hypersensitivity reactions; in agreement with previous studies by Kilshaw and Sissons (1979 a, b). They suspected such an involvement, on the basis of the high circulating anti-soya antibody titres, the positive results of passive cutaneous anaphylaxis tests and the apparent link between the levels of immunoreactive glycinin and β -conglycinin in soya products and the triggering of the intestinal disturbances in calves already sensitised to HSF. The onset of the gut motor disorders less than an hour after an antigen challenge (Lallès et al 1993, Sissons 1993) is also consistent with an immediate mechanism. Since the presence of detectable levels of specific IgE in plasma does not seem to be a constant feature of allergy in calves (Kilshaw and Sissons, 1979 a, b) and infants (Heyman et al 1994), the participation of non-IgE antibodies in the skin reactions is not excluded. While Kunitz protease inhibitors are potent true allergens in BALB/c mice (Shibasaki et al 1980, Ogawa et al 1992), the situation is more complex in human beings because at least 16 allergens have been identified. Plasma IgE was bound principally to the 11S and 7S fractions, although proteins of lower molecular weight were the major allergens in some patients (Shibasaki et al 1980, Burks et al 1988, Ogawa et al 1991). Hydrophobic proteins from soyabean hulls are also important allergens in asthma (Gonzalez et al 1992).

Glycinin and β -conglycinin, but also α -conglycinin, BBI and lectins, induced skin oedema reactions four to eight hours after injection. This is consistent with their hypothetical role in a semi-delayed, type III, hypersensitivity reaction (Barratt et al 1979). In the present work, the implication of a delayed cell-mediated mechanism is strongly supported by the long-term skin thickening and the lymphoproliferation of PBL in vitro in response to β -conglycinin in the calves fed HSF. Previous attempts to demonstrate such reactions remained unsuccessful in the calf (Heppell et al 1987, Dawson et al 1988). However, they were observed in piglets by using skin tests (Stokes et al 1987) and in infants suffering from intolerance to soya (Van Sickle et al 1985) or cow's milk protein (Heyman et al 1994) by specific in

vitro lymphoproliferation tests. Whether this lymphocyte sensitisation is secondary to an abnormally high uptake of antigen by the gut is not known.

Disturbances in gut secretion and motility after an antigen challenge are linked with the specific release of mediators from intestinal mast cells (Crowe and Perdue 1992). Histamine (via H-1 receptors), serotonin (via 5HT-2 receptors), leukotriens and platelet-activating factor (PAF) are implicated in abnormal chloride secretion (Crowe and Perdue 1992), and serotonin (via 5HT-3 receptors) and arachidonic acid derivatives are responsible for motility disorders in rats sensitised to egg albumin (Fargeas et al 1992).

In the present work it was found that histamine was partly responsible via H-1 receptors, for the skin oedema after the intradermal injections. This is in agreement with the observation that the same calves, when pretreated with the H-1 receptor antagonist and then orally challenged with HSF, had a lower incidence of diarrhoea and milder myoelectric complexes migrating along the jejunum than without pretreatment (Lallès et al 1994). In addition to histamine, PAF but not leukotriens or thromboxanes may possibly be implicated in motility disorders in calves (Lallès et al 1994). Finally, these mediators probably originate from mast cells, because Sissons et al (1989) observed an increased number of these cells in the small intestine of calves sensitised to HSF.

Similar skin reactions were developed with raw soya and HSF extracts, indicating that steam-heating did not inactivate the antigens (Kilshaw and Sissons 1979b, Tukur et al 1993). This finding is in agreement with the fact that the thermal denaturation of soya protein extracts did not influence the IgE and IgG-specific binding activities in children sensitive to soya (Burks et al 1992). Steam-heating is usually regarded as sufficient to reduce the biological activities of protease inhibitors and lectins substantially (Sissons 1993), implying that these molecules can still interfere, as allergens, with the animal via its immune system. Moreover, all the purified soya proteins tested here, except glycinin, induced skin reactions in the control calves (Tables 1 and 3). They could be the thermostable factors of soyabean which have been shown to provoke an accumulation of fluid in the small intestine of pigs not sensitised to soya (Nabuurs 1986). Therefore, the 'non-immune' effect of these proteins on the gut may be important in the aetiology of soya sensitivity in young farm animals. Skin oedema reactions did not appear when the soya proteins were hydrolysed, indicating the key role of protein structure in these short-term responses, and the possible value of such a treatment to reduce the antigen levels in soya products (Lallès et al 1995). Although delayed skin reactions were absent with HSPI, PBL from calves fed HSF proliferated in the presence of HSPI in vitro. This latter reaction is consistent with the concept that it is a small peptide which is presented by an antigen-presenting cell to the specific T lymphocyte (Sette and Grey 1992). Thus, such reactive peptide(s) may have been resistant to the hydrolysis treatment. The lack of delayed skin reactions with HSPI could have resulted from the rapid diffusion of the peptides from the site of injection. Alternatively, an immediate reaction could be necessary for the development of a delayed skin thickening (Torii et al 1993).

Many proteins from soyabean, including glycinin, α -conglycinin, β -conglycinin, Bowman-Birk protease inhibitors and lectins may participate in the calf's sensitivity to soya, possibly via type I, III and IV immune mechanisms.

In particular, β -conglycinin was able to stimulate delayed skin reactions and specific lymphoproliferation in vitro. The direct implication of these proteins in the secretory and motor disorders of the gut remains to be established. The study of the interactions between these proteins in the gut, as in those demonstrated in rabbits (Alvarez and Torres-Pinedo 1982) may improve our understanding of the pathophysiology of soya sensitivity in farm animals.

ACKNOWLEDGEMENTS

The authors wish to thank Dr G. Peltre (Pasteur Institute, Paris, France) for stimulating discussions. The staff of the experimental facilities and Mrs J. Quillet are acknowledged for animal care and literature collection, respectively. Part of this work was financially supported by the Ministère de l'Agriculture et de la Pêche (DGER No 91-131).

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Received September 9, 1994
Accepted August 1, 1995