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## IgM, IgA, IgG1 and IgG2 specific responses in blood and gut secretion of calves fed soyabean products

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### Abstract

Calves fed soya proteins may develop severe gastrointestinal disorders. Whether these are predominantly associated with particular Ig subclasses and (or) dietary proteins remains unclear. Therefore, antibody responses to soyabean protein were analysed by dot- and blot-immunobinding in plasma and intestinal mucous secretions. One-month-old calves were fed for 2.5 months liquid diets based on skim milk powder (SMP) or a mixture (2:3, protein basis) of whey and soyabean products including a low antigenic hydrolysed soya protein isolate (HSPI) and a highly antigenic heated soya flour (HSF). Specific antibodies (Abs) of the main isotypes (IgM, IgA, IgG1, IgG2) were characterised by immunostaining of samples which had been previously incubated with nitrocellulose sheets coated with SMP, HSPI or HSF extracts.

Plasma collected before feeding experimental diets showed very little specific Abs. By contrast, 2.5 months later, a three-fold increase ( $P < 0.05$ ) in IgG1 and IgA titres against HSF antigens was observed in calves fed HSF compared with those fed the control or HSPI diet. IgG1 immunoblotting revealed many protein bands from soya in the molecular range of 22–32 and 38–42 kDa. Immunorecognition of specific proteins from SMP and HSPI remained low and similar among animal groups.

Specific IgM, IgA and IgG1 titres against HSF, and to a lesser extent HSPI, were significantly higher ( $P < 0.05$ ) in jejunal mucous secretion of calves fed HSF compared with other groups. Secretions from calves fed HSF bound to many soyabean proteins in the range of 17–23 and 26–38 kDa, with similar patterns for IgA and IgG1. By contrast, only weak bands were found for IgM and IgG2 in all groups of calves.

Thus, calves fed antigenic HSF do present specific Abs including IgG1 and IgA isotypes, both systemically and locally. Therefore, IgG1 and (or) IgA rather than IgM and IgG2 Abs may be preferred for assessing the immunogenicity of soyabean products in calves. Interestingly, soyabean immunogenicity was drastically reduced by adequate proteolysis.

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## 1. Abbreviations

Ab, antibody; ANF, antinutritional factors; SMP, skim milk powder; HSF, heated soya flour; HSPI, hydrolysed soya protein isolate; Ig, immunoglobulin; Mab, monoclonal antibody; MW, molecular weight.

## 2. Introduction

Soyabean proteins are commonly incorporated in milk replacers for veal calves (Kolar and Wagner, 1991). However, soyabean products improperly treated may lead to gastrointestinal adverse reactions characterised by alterations in gut morphology and physiology, diarrhoea and poor growth (review by Sissons, 1989; Lallès et al., 1993a). Many factors including animal, dietary and environment as well as intestinal bacterial flora have been suspected in this food sensitivity. Adverse reactions may be related to major soyabean storage globulins, glycinin and  $\beta$ -conglycinin (Sissons, 1982), or usual antinutritional factors (ANF) like protease inhibitors and lectins (review by Huisman and Jansman, 1991). However, even if these ANF were inactivated by steam heating, gut disturbances were still observed (Sissons, 1982). Indeed, only specific treatments including hot-ethanol denaturation or enzymatic proteolysis appeared more satisfactory to improve calf performance (Sissons et al., 1982).

An immunological basis has been suspected for this intolerance (review by Sissons, 1982). A local IgA deficiency was also suggested to account for calves' susceptibility to soya (Barratt and Porter, 1979). Whether local and systemic variations in the specific Ig isotypes were associated with antigen levels in soyabean products, as observed for bacterial or viral infection (Heckert et al., 1991), remains unknown.

In a recent study conducted in calves, poor growth and high plasma antisoya antibodies appeared to be linked with the consumption of a high antigenic heated soya flour (Lallès et al., 1994). These effects were virtually absent when the proteins from soya were enzymatically hydrolysed. In the present work, we compare quantitatively and qualitatively the specific antibodies (IgM, IgA, IgG1, and IgG2) found in blood and mucosal secretions of these calves. Preponderant IgG1 and IgA responses were found in both fluids from the calves fed the highly antigenic soya flour. Moreover, numerous soyabean proteins were recognised by antibodies present in these calves but not in the calves fed the low antigenic hydrolysate or the control diet.

## 3. Materials and methods

### 3.1. Soya products

One soya product was a hydrolysed soya protein isolate (HSPI) provided by Protein Technologies International (St Louis, MO, USA). The second was a heated soya flour

(HSF) from Société Industrielle des Oléagineux (Bourgival, France). Antigenicity of the protein sources was tested by enzyme-linked immunosorbent assay (ELISA), and glycinin and  $\beta$ -conglycinin were detected in HSF only (Lallès et al., 1994).

### *3.2. Animals and diets*

Experiments were conducted on three groups of seven male Holstein calves. They were fed milk replacers containing skim milk protein (SMP) or a mixture (2:3) of whey and either a non-antigenic hydrolysed soya protein isolate (HSPI) or an antigenic heated soya flour (HSF) from 32 to 135–145 days of age. The analyses described here were conducted on three, four and five calves randomly chosen in the SMP, HSPI and HSF groups, respectively. Details of the experiments have been published elsewhere (Lallès et al., 1994).

### *3.3. Samples*

Plasma was collected before (Day 25) and 2.5 months after (Day 100) the start of feeding experimental diets, and then stored at  $-20^{\circ}\text{C}$ . Immediately after slaughter (Days 135–145), i.e. 4 months after feeding of experimental diets, mucosal secretions from a portion of jejunum of about 20 cm length were collected and stored at  $-80^{\circ}\text{C}$ . The secretions were gently scraped from the mucosa with a glass slide, taking care to limit contamination with blood. They were treated at  $4^{\circ}\text{C}$  as described by Newby et al. (1979). Briefly, proteins from mucosal secretions were extracted in acetate buffer (pH 4.5, 10 mM) containing 0.1% (w/v) SBTI, 5 mM EDTA to inhibit enzymatic reactions and  $80\ \mu\text{g ml}^{-1}$  of gentamicin,  $10\ \text{UI ml}^{-1}$  penicillin and  $10\ \mu\text{g ml}^{-1}$  of streptomycin to avoid bacterial contamination. All the chemicals were supplied by Sigma. Then, the mixture (mucus/acetate buffer: 1/5) was sonicated (Branson, Danbury, CT, USA). Proteins including immunoglobulins were recovered in supernatants after centrifugation ( $40\ 000\times g$ , 1 h). Supernatants were dialysed against phosphate buffer (150 mM, pH 7.4) overnight. Finally, extracts were freeze-dried and stored at  $-20^{\circ}\text{C}$  until use.

### *3.4. Antigen extracts*

Three soluble antigen mixtures including SMP (negative control) and extracts from HSPI and HSF were prepared as follows. Each product was incubated (w/v: 1/10) for 1 h at room temperature in a Tris-HCl buffer (100 mM, pH 8). Then, the mixture was centrifuged for 30 min at  $18\ 000\times g$  and  $15^{\circ}\text{C}$ . Extracts were then stored at  $-20^{\circ}\text{C}$ . Additionally, an extract was prepared similarly from raw defatted soybeans and served as antigen in blot-immunobinding studies. Antigen solutions were used at  $1\ \text{mg ml}^{-1}$  in immunobinding studies. The protein concentration of the supernatant was determined according to Lowry et al. (1952).

### *3.5. Antibodies*

Monoclonal antibodies (Mab) against bovine IgA and IgG1 (Van Zaane and Ijzerman, 1984), were kindly provided by Dr A. Bianchi (Central Veterinary Institute, Lelystad, the

Netherlands) and used at a 1:5000 dilution. Mabs against bovine IgG2 and IgM were obtained from Sigma and used at a 1:500 dilution. In dot-immunobinding studies, sheep polyclonal monospecific antibodies against bovine IgA and IgG1 isotypes, and conjugated with peroxidase (Bethyl Laboratories; Montgomery, TX, USA) were also used since no difference was found when compared with monoclonal antibodies provided by Dr A. Bianchi.

### *3.6. Dot-immunobinding assay*

Dot-immunobinding assays were used to test specific soyabean responses in plasma and mucosal secretion extracts. A wet membrane (Hybond C+, Amersham International, UK) was placed in a dot apparatus (Convertible Filtration Manifold System, Gibco BRL, Gaithersburg, MD, USA). Each of the 96 wells received 5 ml of a given antigen solution ( $1 \mu\text{g ml}^{-1}$ ). After 10 min of incubation under vacuum, membranes were blocked with Tris–milk buffer (skim milk powder (5%) in Tris buffer (Tris 10 mM, NaCl 150 mM, pH 8.0) for 30 min. When SMP was the coating antigen, gelatin 2% (Sigma) in Tris buffer was used as the blocking solution. After washing in a Tris–Tween 20 buffer (0.5% Tween 20 (Sigma) in Tris buffer), membranes were replaced in the same position in the dot apparatus. Then, serial doubling dilutions of the plasma or mucosal secretion extracts in phosphate buffered saline (PBS, 150 mM, pH 7.2) were added to each well. After 10 min of binding, membranes were washed in Tris–Tween 20 buffer. All the following steps were carried out in batches.

After a rapid wash with 150 ml of Tris–Tween 20 buffer, specific bovine antibody isotypes were revealed by different Abs against IgM, IgA, IgG1 and IgG2 in Tris–Tween buffer. Specific bands were detected by a peroxidase-conjugate antibody (Jackson Immuno-Research Laboratories, West Grove, PA, USA). Diaminobenzidine (Sigma) was used as the enzyme substrate. Results were recorded as titres, e.g. numbers of doubling dilutions from the initial dilution beyond which dot signals were no longer apparent on the membranes.

### *3.7. Blot-immunobinding assay*

The western blot system was used to identify soya proteins detected by the different isotypes of calf immunoglobulins in blood and gut secretions. The blot-immunobinding applied to biological fluids in pigs has been described previously (Dréau et al., 1994). Briefly, 0.5 mg of the soyabean protein extract was applied to the system and run in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970 as modified by Devine and Birrell, 1992). Proteins were transferred from the gel to a nitrocellulose membrane (Hybond C+) using a transblot apparatus (Pharmacia, Uppsala, Sweden). Membranes were dried and stored at 4°C until use.

The blocking step was achieved in Tris milk buffer. Membranes bearing separated antigens were incubated with 50  $\mu\text{l}$  of the different calf plasma or mucosal secretion extracts during 45 min in a multichannel miniblottedter 28S (Immunogenetics, Cambridge, MA, USA) as described by Hoff et al. (1988). The following steps including Ig isotype detection and revelation were carried out in batches, as described above for dot-immunobinding studies.

### 3.8. Statistical analysis

Statistical analyses were performed using the ANOVA program (Abaccus Concept, Berkeley, CA, USA). Variation in time of specific antibody levels tested for each coating antigen within group, and dietary treatment effect were analysed by ANOVA. When significant ( $P < 0.05$ ) diet effects were recorded, differences between groups for Ab titres were compared using the Scheffé's test.

## 4. Results

### 4.1. Anti-soya antibodies in blood

No significant differences ( $P > 0.10$ ) between antibody levels against SMP, HSPI and HSF antigens were found before the beginning of the experiment in plasma from the three animal groups, regardless of Ig isotype (data not shown). Two and a half months later, anti-SMP antibody titres remained unchanged in the three groups of calves whilst antibody titres of the IgA, IgG1 and IgG2 isotypes against HSPI and HSF antigens were significantly higher than before starting to feed the experimental diets ( $P < 0.05$ , Table 1). The highest increase in IgA and IgG1 isotypes was observed against HSF antigens ( $P < 0.001$ , Table

Table 1

Antibody titres ( $\log_2$  dilution, means  $\pm$  SEM) obtained by dot-immunobinding for the different isotypes tested (IgM, IgA, IgG1 and IgG2) and for skim milk powder (SMP), hydrolysed soya protein isolate (HSPI), heated soya flour (HSF) with sera collected after 75 days of treatment (Day 100) in calves fed skim milk powder (SMP), hydrolysed soya protein isolate (HSPI) or heated soya flour (HSF) for 4 months

Coating antigen	Antibody isotype	Dietary treatment			$P^a$
		SMP ( $n = 3$ )	HSPI ( $n = 4$ )	HSF ( $n = 5$ )	
SMP	IgM	7.3 $\pm$ 0.3	8.2 $\pm$ 0.5	8.8 $\pm$ 0.4	0.10
	IgA	0a $\pm$ 0	0.2a $\pm$ 0.2	1.6b $\pm$ 0.2	0.0015
	IgG1	0a $\pm$ 0	0a $\pm$ 0	0.8b $\pm$ 0.2	0.0044
	IgG2	4.0 $\pm$ 0.0	4.2 $\pm$ 0.5	4.6 $\pm$ 1.1	0.24
HSPI	IgM	7.7 $\pm$ 0.3	7.2 $\pm$ 0.2	7.0 $\pm$ 0.3	0.37
	IgA*	1.0ab $\pm$ 0.0	0.5a $\pm$ 0.5	2.0b $\pm$ 0.3	0.0369
	IgG1*	2.3a $\pm$ 0.3	4.7ab $\pm$ 0.7	6.0b $\pm$ 0.5	0.0085
	IgG2*	2.0a $\pm$ 0.6	3.2a $\pm$ 0.2	5.0b $\pm$ 0.3	0.0009
HSF	IgM	8.3 $\pm$ 0.3	8.0 $\pm$ 0.0	7.4 $\pm$ 0.4	0.18
	IgA***	0.7a $\pm$ 0.7	0.7a $\pm$ 0.7	6.2b $\pm$ 0.2	0.0001
	IgG1***	3.3a $\pm$ 0.9	4.0a $\pm$ 0.4	9.8b $\pm$ 0.4	0.0001
	IgG2***	2.0a $\pm$ 0.0	3.2a $\pm$ 0.2	6.4b $\pm$ 0.6	0.0002

<sup>a</sup>Probability obtained by ANOVA analysis.

Different letters after means indicate significant differences between groups ( $P < 0.05$ , Scheffé's test).

\* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.001$ : ANOVA analysis of differences in time (before Day 25 and after Day 100) 2.5 months of feeding the respective diets) for given coating antigen and isotype.

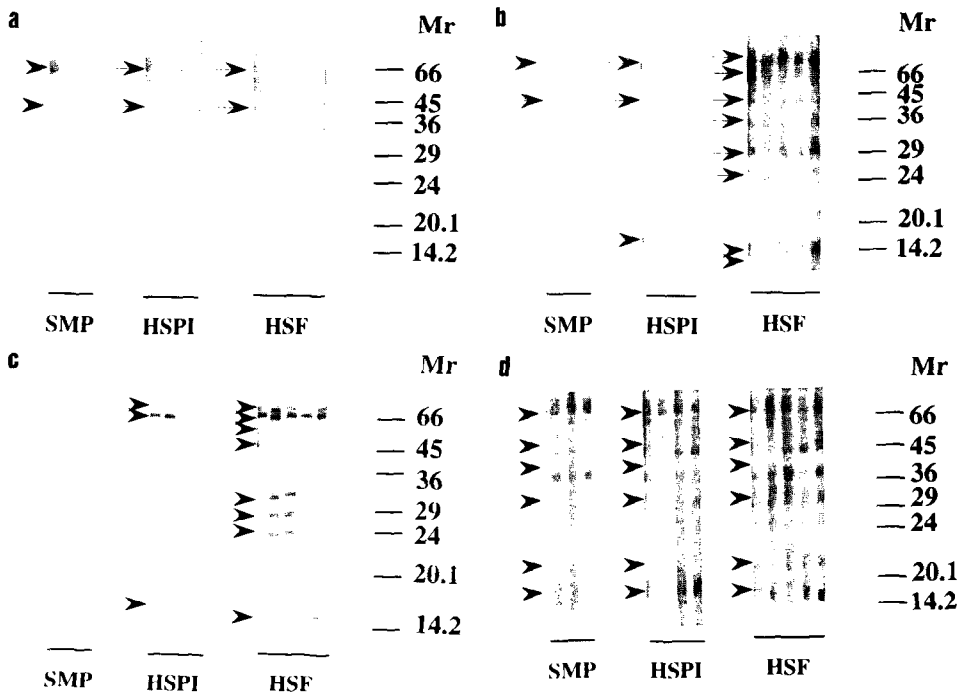


Fig. 1. Blot-immunobinding patterns of specific IgM (a), IgA (b), IgG1 (c) and IgG2 (d) obtained against an extract of raw soya proteins (RSF) with plasma (dilution 1:2 except for IgG1 1:20) from 100-day-old calves fed for 4 months with skim milk powder (SMP,  $n=3$ ), hydrolysed soya protein isolate (HSPI,  $n=4$ ) or heated soya flour (HSF,  $n=5$ ). Each lane represents a calf. Arrows indicate specific bands present in the majority of calves, in a given group.

1). IgM was not significantly different between groups despite high absolute titres, whatever the coating antigen (Table 1). Animals fed SMP or HSPI had antibody titres against HSPI antigens which were low and not significantly different from each other ( $P > 0.05$ ). By contrast, corresponding titres in the calves fed HSF were higher than in the other groups ( $P < 0.05$ , Table 1). Moreover, specific IgA levels were four-fold higher in HSF fed calves than in those fed HSPI ( $P < 0.05$ , Table 1). For HSF antigens, antibody titres of the IgA and IgG1 isotypes were significantly higher in the HSF group than in the other groups ( $P < 0.05$ , Table 1). Specific IgG2 levels were also higher in the HSF group than in the SMP group ( $P < 0.05$ , Table 1).

Identification of specific soyabean proteins recognised by these circulating antibodies after 2.5 months of treatment was conducted by blot-immunobinding. Apart from the IgM isotype for which the binding was weak, bands from the RSF extract were much more immunostained with plasma from calves fed HSF than from other animals, particularly for IgA and IgG1 isotypes (Figs. 1(b) and 1(c)). Concerning IgA, only plasma from the HSF group recognised bands of the soyabean protein extract (Fig. 1(b)). The major bands recognised by IgA had molecular weights (MWs) of about 15, 32, 36, 43 and 69 kDa (Fig. 1(b)). Patterns for IgG1 antibodies also differed between the three groups of calves (Fig. 1(c)). The IgG1 from plasma of calves fed HSF were strongly directed against almost all

Table 2

Antibody titres ( $\log_2$  dilution, means  $\pm$  SEM) obtained by dot-immunobinding for the different isotypes tested (IgM, IgA, IgG1 and IgG2) and for skim milk powder (SMP), hydrolysed soya protetin isolate (HSPI), heated soya flour (HSF) with mucosal secretions collected after slaughter (Days 135–145) in calves fed skim milk powder (SMP), hydrolysed soya protein isolate (HSPI) or heated soya flour (HSF) for 4 months

Coating antigen	Antibody isotype	Dietary treatment			<i>P</i> <sup>a</sup>
		SMP ( <i>n</i> =3)	HSPI ( <i>n</i> =4)	HSF ( <i>n</i> =5)	
SMP	IgM	3.7a $\pm$ 0.3	4.2ab $\pm$ 0.5	5.6b $\pm$ 0.4	0.0278
	IgA	3.7 $\pm$ 0.8	3.5 $\pm$ 0.8	5.8 $\pm$ 0.4	0.0580
	IgG1	4.0 $\pm$ 0.6	4.0 $\pm$ 0.7	4.6 $\pm$ 0.2	0.6149
	IgG2	4.3 $\pm$ 0.3	5.0 $\pm$ 0.4	4.6 $\pm$ 0.2	0.43
HSPI	IgM	3.0a $\pm$ 0.6	2.0a $\pm$ 0.0	4.8b $\pm$ 0.4	0.0009
	IgA	3.7a $\pm$ 0.6	5.7ab $\pm$ 0.6	5.8b $\pm$ 0.2	0.0276
	IgG1	3.7a $\pm$ 0.6	4.0a $\pm$ 0.7	6.2b $\pm$ 0.2	0.0112
	IgG2	2.7 $\pm$ 0.3	3.7 $\pm$ 1.1	4.8 $\pm$ 0.6	0.23
HSF	IgM	2.0a $\pm$ 0.6	2.5a $\pm$ 0.6	5.0b $\pm$ 0.5	0.0117
	IgA	6.3a $\pm$ 0.8	7.2a $\pm$ 1.4	14.0b $\pm$ 1.0	0.0016
	IgG1	3.0a $\pm$ 0.6	4.7a $\pm$ 1.0	9.6b $\pm$ 0.8	0.0012
	IgG2	3.0 $\pm$ 0.6	3.7 $\pm$ 1.1	5.6 $\pm$ 0.4	0.0832

<sup>a</sup>Probability obtained by ANOVA analysis.

Different letters after means indicate significant differences between groups ( $P < 0.05$ , Scheffé's test).

the bands of the soya protein extract (Fig. 1(c)). A specific IgG1-binding to proteins of MWs between 22 and 32 kDa or between 38 and 42 kDa was observed with plasma from calves fed HSF but not in the other groups of calves (Fig. 1(c)). Qualitative immunostaining observed with plasma from animals fed HSPI was usually weak, regardless of antigen mixture. Calves fed SMP presented antibodies which recognised only a few bands of RSF with usually weak patterns (Fig. 1(c)). No apparent qualitative difference between the three groups was observed in the IgG2 responses against RSF for which six major bands with MWs of about 16, 22, 31, 39, 44, 68 kDa were revealed (Fig. 1(d)).

#### 4.2. Antisoya antibodies in mucosal secretions

No difference was found by dot-immunobinding between animal groups for Abs against SMP antigens, except for IgM whose titre was higher in the mucus of animals fed HSF compared to others (Table 2). For HSPI antigens, Ab levels were significantly higher ( $P < 0.05$ ) in calves fed HSF compared with controls, for IgM, IgA, and IgG1 isotypes (Table 2). Antibody responses for IgA and IgG1 isotypes were much higher ( $P < 0.05$ ) against HSF antigens in mucosal secretions collected from animals fed HSF compared with other groups (Table 2). IgA titres against HSF extracts were higher in animals fed HSF than in the other groups ( $P < 0.05$ , Table 2). No difference between groups was observed for IgG2 isotype regardless of antigens tested (Table 2).

Blot-immunobinding analysis of mucous samples for specific Abs against the soyabean protein extract was in general agreement with observations made on plasma samples (Fig.



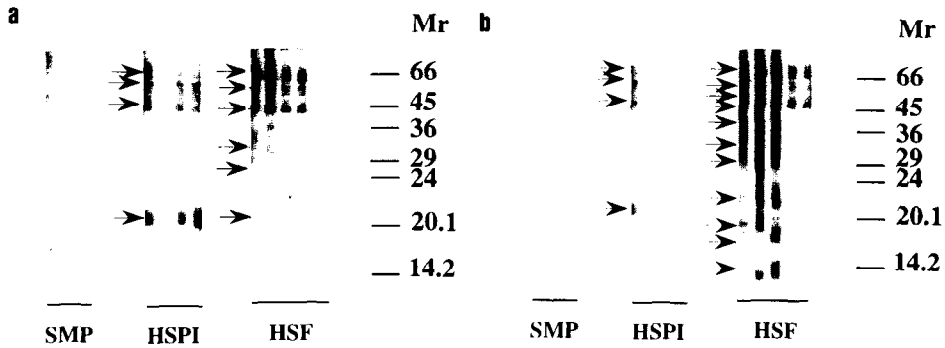


Fig. 2. Blot-immunobinding patterns of specific IgA (a), and IgG1 (b) obtained against an extract of raw soya proteins (RSF) with mucosal secretion extracts (dilution 1:5) from 135–145 day old calves fed for 4 months with skim milk powder (SMP,  $n = 3$ ), hydrolysed soya protein isolate (HSPI,  $n = 4$ ), or heated soya flour (HSF,  $n = 5$ ). Each lane represents a calf. Arrows indicate specific bands present in the majority of calves, in a given group.

2). In both groups of calves fed soyabean proteins, two to three soya proteins were weakly bound, as far as IgM and IgG2 were concerned (data not shown). IgA recognised bands of MW 45, 54 and 67 kDa from the RSF extract in mucosal secretions of the calves fed HSPI or HSF (Fig. 2(a)). One calf of the SMP group also recognised these bands (Fig. 2(a)). Additionally, IgA in calves fed HSPI and to a lesser extent those fed HSF recognised a band of about 22 kDa. Numerous bands from 27 to 36 kDa and from 47 to 60 kDa were revealed by IgA with mucus from calves fed HSPI and HSF (Fig. 2(a)). Concerning the IgG1 pattern, no band was observed with the mucosal secretions of calves fed SMP (Fig. 2(b)). Mucosal IgG1 from calves fed HSF recognised a lot of bands in the RSF extract. The major stained bands had MWs of approximately 14, 18, 19, 22, 30, 34, 38, 47, 54, 59 and 68 kDa. Moreover, bands of low MWs (less than 12 kDa) and bands between 38 and 40 kDa were revealed by IgG1, but not by IgA antibodies, present in mucosal secretions of calves fed HSF.

## 5. Discussion

Our results showed significantly higher IgA and IgG1 specific antibody titres at both systemic and local levels in calves fed high antigenic compared with those fed low antigenic soya products. Moreover, numerous protein bands from soya were qualitatively recognised by Abs present in plasma and mucosal secretions. The systemic antibody response to feeding heated soya products with high antigenicity reached high levels in calves in agreement with earlier work on this subject (Barratt and Porter, 1979; Kilshaw and Sissons, 1979). This increase depended on the way soya products were treated as previously shown (review by Sissons, 1982). Indeed, the more glycinin and  $\beta$ -conglycinin were denatured, the more soya products were suitable for calf feeding.

Differences observed here in specific IgG and especially IgG1 levels have already been reported in the blood of calves fed heated soya (Heppell et al., 1989; Mir et al., 1993). Additionally, our results showed an important IgA response. By contrast, previous works

suggested IgG1 (Newby and Bourne, 1976) or IgA (Porter et al., 1972; Barratt and Porter, 1979) deficiencies in mucosal secretions of calves. However, increases in specific IgG1 and IgA levels, as observed here, were the most obvious features in infants suffering from gluten intolerance (Kelly et al., 1991). Nevertheless, high levels of specific IgA and IgG1 in mucosal secretions were not sufficient to prevent the gut disturbances in the calves fed HSF.

Disorders in intestinal absorption and motility in calves already sensitised to antigenic soya proteins are usually observed 1–3 h after soya challenge (Sissons, 1982; Sissons and Tolman, 1991; Lallès et al., 1993b), and may refer to immediate hypersensitivity reactions (Van Dijk et al., 1988). Recent studies suggested that antigens inducing IgG1 response first also involved IgE response in a second time, depending on the antigen levels (Snapper et al., 1988; Armerding et al., 1993). Specific IgE can be assayed using the anti-bovine IgE Mab developed by Thatcher and Gershwin (1988). Such an IgE response was not evidenced here by peroxidase immunostaining, possibly because IgE levels were low (data not shown). But a contribution of IgE antibodies in the hypersensitivity to soyabean proteins has been shown by passive cutaneous anaphylaxis in a limited number of calves (Barratt and Porter, 1979; Kilshaw and Sissons, 1979). More direct evidence of individual IgE responses and allergen identification are currently under investigation using radiolabelled antibody probes.

At the local level, we have already shown alterations of the mucosa including a reduction of villus height in calves fed HSF (Lallès et al., 1994), in agreement with previous studies (Kilshaw and Slade, 1982). In piglets fed antigenic soya, such alterations were also observed (Miller et al., 1991; Dréau et al., 1994), and the involvement of cellular immune reactions was suggested (Miller et al., 1991). Thus, in calves, alterations of the villi could also result from a cellular hypersensitivity reaction. This hypothesis seems to be supported by the cellular infiltration observed locally in calves fed HSF and the significant *in vitro* proliferation of lymphocytes to some purified soya proteins (Dréau et al., unpublished data, 1993).

Soya proteins with MWs of about 20–23, 30–32, and 37–42 kDa were detected by IgG1 and IgA Abs from calves fed HSF only. The band of 30–32 kDa may correspond to the subunit of soyabean agglutinin (Yates and Sage, 1983). The bands of 20–23 and 37–42 kDa were similar to glycinin acid and basic subunits, respectively (Nielsen, 1985). These bands were only observed in calves fed HSF in both plasma and mucosal secretions. Other specific bands of 47–51, 59–62 and 65–68 kDa may correspond to the three subunits of  $\beta$ -conglycinin (Winters et al., 1990). They were detected in calves fed HSPI or HSF. Moreover, visual differences between groups were observed in the staining intensity of bands corresponding to glycinin and  $\beta$ -conglycinin, suggesting that these proteins were the most antigenic components of soyabean proteins (Kilshaw and Sissons, 1979; Sissons, 1982). Other non-identified bands were also recognised by plasma and mucus Abs from calves fed HSF or HSPI. In addition, bands below 15 kDa were recognised only by IgG1 from the calves fed HSF, strongly suggesting that antigens of these MWs could also be part of the hypersensitivity reaction. A band of about 22 kDa was detected by IgG2 probing in the calves fed HSPI but it was not observed in the calves fed HSF. This may indicate that hydrolysis of proteins is responsible for the appearance of neo-epitopes. HSPI probably shares common epitopes with HSF, but these epitopes may not be deleterious since growth (Lallès et al., 1994) or gut myoelectric activity (Lallès et al., 1993b) were near-normal with HSPI.

This study leads to the conclusions that soya intolerance in calves may not be linked with IgA or IgG1 immunodeficiencies, and that a lot of potential antigens from soyabean products may be suspected in gastrointestinal disorders. The semi-quantitation of antibody responses with regard to specific antigens and Ig isotypes is under investigation. Finally, experiments on specific IgE, interleukins and antigens/allergens may provide new lights on dietary hypersensitivity reactions in farm animals.

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