

**Systemic and local antibody responses of preruminant calves to proteinaceous antinutritional factors from the soyabean.**

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Sixteen male 3-week-old Holstein calves were fed milk replacers based on skim-milk powder (SMP,  $n = 8$ ) or a mixture (70:30, CP basis) of heated soya flour (HSF,  $n = 8$ ) and whey for 2–3 months. Serum and jejunal mucous secretions were collected after 10 weeks of experiment and at slaughter, and were analysed by dot-blotting for IgM, IgG<sub>1</sub>, IgG<sub>2</sub> and IgA antibodies (Abs) against raw soya flour (RSF), HSF, hydrolysed soya protein (HSP), SMP, the major storage globulins glycinin and  $\beta$ -conglycinin,  $\alpha$ -conglycinin, Bowman–Birk protease inhibitors (BBI) and lectins. The HSF calves had previously been shown to have short- and long-term soya-positive skin tests, gut motility disorders and diarrhoea. Differences between calf groups for serum-specific IgM were usually not significant, except with RSF ( $P < 0.05$ ), due to high background titres in controls. In this group, serum titres were below 1 for other isotypes. In HSF calves, IgG<sub>1</sub>, IgG<sub>2</sub> and IgA serum titres of 12, 7 and 8, respectively were observed against RSF and HSF; with HSP, IgG<sub>1</sub> titres were below 3 and the other Ig titres were below 1; and all serum Ig titres were below 1 with SMP. Calves fed HSF produced significant levels of serum IgG<sub>1</sub> (titres 3.5–7) against all the purified soya proteins, serum IgG<sub>2</sub> against  $\alpha$ -conglycinin,  $\beta$ -conglycinin, and BBI (titres 2–5), and serum IgA against all but  $\alpha$ -conglycinin (titres 2.5–4.6). Specific Abs titres in jejunal mucous secretions were significantly higher ( $P < 0.05$ – $0.01$ ) in calves fed HSF than in controls for IgM, IgG<sub>1</sub> and IgG<sub>2</sub> against most antigens including HSP and, to a much lesser extent, SMP. This was also true for IgA although differences reached significance for  $\beta$ -conglycinin and lectins only. In conclusion, calves sensitised to HSF do produce systemic and local antibodies against most proteinaceous antinutritional factors from soya including protease inhibitors and lectins. IgA production was also noticeable, excluding IgA deficiency as a possible aetiological factor to soya hypersensitivity in young calves. IgE responses remain to be established for identifying true allergens.

**Identification of soyabean allergens in the preruminant calf: specific skin and lymphoproliferation test responses.**

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Twenty-two male 3-week-old Holstein calves were fed milk replacers based on skim-milk powder (SMP,  $n = 14$ ) or a mixture (70:30, CP basis) of heated soya flour (HSF,  $n = 8$ ) and whey for 2 months. Intradermal injections of raw soya flour (RSF) HSF, hydrolysed soya protein (HSP), SMP, the major storage globulins, glycinin and  $\beta$ -conglycinin,  $\alpha$ -conglycinin, Bowman–Birk protease inhibitors (BBI), lectins and adequate positive and negative controls were conducted with or without antihistamine premedication. Skin oedema diameter (SOD) and double skin-fold thickness (DSFT) were recorded up to 8 h and 5 d after injection, respectively. Lymphoproliferation tests were also carried out on peripheral blood leucocytes (PBL) cultured for 5 d with either HSF or HSP saline extracts, purified glycinin or  $\beta$ -conglycinin, or adequate controls. Calves fed HSF exhibited significant ( $P < 0.05$ – $0.01$ ) skin oedema reactions between 1 and 8 h post-injection against all the proteins tested (including SMP at 1 h) except HSP. These reactions were largely reduced under anti-H1 histamine receptor antagonist. DSFT was significantly ( $P < 0.05$ – $0.01$ ) increased at sites of injection of RSF and HSF (d 1 and d 4),  $\alpha$ -conglycinin (d 1), and  $\beta$ -conglycinin principally (d 1 to d 5). PBL cells cultured from SMP and HSF-fed calves for 5 d exhibited similar responses to non-dietary mitogens. PBL from control calves with and without soya protein showed a similar low proliferation. In contrast, PBL from HSF calves presented dose-dependent proliferative responses to HSF and HSP extracts, to  $\beta$ -conglycinin but not to glycinin. It is concluded that most proteinaceous antinutritional factors from soya are probably implicated in immediate and semi-delayed hypersensitivity reactions in the young calf. Delayed, eg T-lymphocyte-mediated, reactions may be primarily due to  $\beta$ -conglycinin as judged from both skin and lymphoproliferation tests. Heating soya products did not reduce skin reactions while proteolysis did. However, T-cell reactive peptides may survive the latter treatment.