Reprod. Nutr. Dev. 39 (1999) 455–466

Original article

Morphology and enzyme activities of the small intestine are modulated by dietary protein source in the preruminant calf

Lucile Montagne[a], René Toullec[a], Tor Savidge[b], Jean-Paul Lallès[a]*

[a] Laboratoire du jeune ruminant, Inra, 65, rue de Saint-Brieuc, 35042 Rennes cedex, France
[b] Institute of Child Health, University of Birmingham, Birmingham B4 6NH, UK

(Received 15 October 1998; accepted 17 May 1999)

Abstract — A study was undertaken to assess the impact of the protein nature and soya antigenicity on the morphology and some enzyme activities of the jejunum in preruminant calves. Twenty Holstein calves fitted with a duodenal cannula were fed a liquid diet based on skimmed milk powder (SMP) for 2 weeks. They were then switched onto diets containing a mixture of SMP and either antigenic heated soybean flour (HSF; $n = 12$) or hypo-antigenic soya protein concentrate (SPC; $n = 8$) for 8 weeks, after which they were reverted back to the SMP diet for 2 weeks. The diets contained similar amounts of digestible nitrogen and energy, and were fed at a rate of 55 g DM/kg$^{0.75}$/d. Proximal jejunal biopsies were collected just before (week 0), during (weeks 2 and 8) and after (week 10) feeding of the soya-based diets, and were used for morphology measurements and the determination of total alkaline phosphatase, lactase, amino-peptidases A and N, and dipeptidyl peptidase IV activities. Feed intake and growth were similar between the HSF and SPC groups during the experimental period. The effects of antigenicity and the antigenicity × time interaction were never significant ($P > 0.05$). Villus height decreased ($P < 0.01$) between weeks 0 and 2, and increased ($P < 0.05$) between weeks 8 and 10. Villus width increased between weeks 2 and 8 ($P < 0.001$). Crypt depth also increased between weeks 0 and 2 ($P < 0.001$). Specific activities of alkaline phosphatase ($P < 0.01$) and amino-peptidase N ($P < 0.05$) decreased between weeks 0 and 2. Conversely, those of alkaline phosphatase ($P < 0.0001$), lactase ($P < 0.01$) and dipeptidyl-peptidase IV ($P < 0.0001$) increased between weeks 8 and 10. Specific activities for lactase and amino-peptidase N decreased ($P < 0.01$) between weeks 2 and 8. The treatments had little effects on the amino-peptidase A activity. In conclusion, the present work demonstrated that soybean protein markedly depressed the morphology and most enzyme activities of the calf small intestine. On the contrary, the in vitro antigenicity of soybean protein had little influence on these parameters in this study. © Inra/Elsevier, Paris.

calf / enzyme / nutrition / small intestine / soybean protein

Résumé — La morphologie et les activités enzymatiques de l'intestin grêle sont modifiées par la nature des protéines alimentaires chez le veau préruminant. Les effets de la nature des protéines

* Correspondence and reprints
E-mail: lalles@roazhon.inra.fr

et de l'antigénicité du soja sur la morphologie et l'activité de quelques enzymes du jéjunum ont été étudiés chez le veau préruminant. Vingt veaux Holstein munis d'une canule duodénale ont consommé un lait à base de poudre de lait écrémé (SMP) pendant 2 semaines, puis ils ont reçu des aliments d'allaitement à base de farine de soja chauffé et antigénique (HSF ; $n = 12$) ou de concentrat de protéines de soja hypo-antigénique (SPC ; $n = 8$) pendant 8 semaines, et ils sont enfin revenus à l'aliment SMP pendant 2 semaines. Les aliments contenaient des teneurs voisines en matières azotées et en énergie digestibles. Ils ont été distribués à raison de 55 g MS/kg^{0,75}/j. Deux biopsies de la muqueuse du jéjunum proximal ont été collectées juste avant (semaine 0), pendant (semaines 2 et 8) et après (semaine 10) la période de distribution des aliments soja. Une biopsie a été fixée dans le formol pour étudier la morphologie, et l'autre a été congelée à –80 °C pour déterminer les activités enzymatiques. La consommation d'aliment et la croissance ont été voisines dans les groupes HSF et SPC. Les effets de l'antigénicité et l'interaction antigénicité × temps n'ont jamais été significatives ($p > 0, 05$). La hauteur des villosités a diminué ($p < 0,01$) entre les semaines 0 et 2, et a augmenté ($p < 0,05$) entre les semaines 8 et 10. Les villosités se sont élargies ($p < 0,001$) entre les semaines 2 et 8. La profondeur des cryptes a aussi augmenté ($p < 0,001$) entre les semaines 0 et 2. Les activités spécifiques de la phosphatase alcaline ($p < 0,01$) et de l'amino-peptidase N ($p < 0,05$) ont diminué entre les semaines 0 et 2. Inversement, celles de la phosphatase alcaline ($p < 0,0001$), de la lactase ($p < 0,01$) et de la dipeptidyl-peptidase IV ($p < 0,0001$) ont augmenté entre les semaines 8 et 10. Les activités spécifiques de la lactase et de l'amino-peptidase N ont diminué ($p < 0,01$) entre les semaines 2 et 8. Les traitements ont eu peu d' effet sur l'activité de l'amino-peptidase A. En conclusion, le présent travail démontre que les protéines de soja ont eu un effet dépressif marqué sur la morphologie et la plupart des activités enzymatiques de l'intestin grêle du veau préruminant. En revanche, l'antigenicité in vitro des protéines du soja a eu peu d'effet sur ces paramètres dans cette étude. © Inra/ Elsevier, Paris.

veau / enzyme / nutrition / intestin grêle / protéine de soja

1. INTRODUCTION

There is a growing demand to substitute skimmed milk powder (SMP) with plant protein, including soybean, in milk replacers for calves. A high incorporation rate of plant protein, however, results in a decreased apparent nitrogen (N) digestibility [15]. The antigenicity and anti-nutritional activities of soybean are also important factors to consider. The digestibility of soybean nitrogen in calves was indeed found to be negatively correlated with the concentrations of immunoreactive glycinin, α-conglycinin and β-conglycinin, and with the anti-tryptic activity in these products [18]. Under our experimental conditions, in which soybean provided between 58 and 71 % of dietary crude protein (CP), the digestibility of soybean N was best predicted by the levels of immunoreactive β-conglycinin, or by the anti-tryptic activity when the products were devoid of β-conglycinin. Highly antigenic products, such as heated soybean flour, presented the lowest digestibility, which could be partially explained by an increased flow of undigested globulins in the ileum [37]. The intrinsic resistance of legume seed protein to digestion [23] is probably the major explanation for a decreased overall digestibility of nitrogen. Pancreatic insufficiency cannot be implicated in calves because feeding soybean protein concentrate (SPC) extracted with hot aqueous ethanol stimulated the pancreatic secretion of trypsin during the first 5 h after the meal, even though the daily trypsin output was similar for both SMP and SPC diets [19]. The intestinal breakdown of peptides by brush border enzymes, and the absorption of oligopeptides and amino acids (AA), may, however, be additional factors limiting the digestibility of soybean proteins. Oligopeptides and AA have been shown to accumulate in the ileum of pigs fed hydrolysed casein [21].

Heated soybean flour (HSF) processed thermally and insufficiently can induce immune-mediated gut disorders in predis-

posed calves. Immunogenic proteins are numerous although β-conglycinin appears to be the most allergenic [19]. As a consequence, villus atrophy and increased densities of T and B lymphocytes in the mucosa can be observed [18]. Information regarding the brush border enzyme activities of intestinal tissue is, however, lacking under such conditions and is an important feature to investigate, because alterations may lead to a malabsorption state.

Small intestinal tissues are usually modified when skimmed milk powder is replaced by soybean products, an observation which may result from simultaneous differences between treatments in feed intake, diet digestibility and soybean in vitro antigenicity. Therefore, the aim of the present work was to demonstrate the negative impact of soybean protein on the morphology and enzyme activities of the proximal jejunum in preruminant calves. Also, two soybean products with different in vitro antigenicity were compared in order to determine the additional effects of this parameter which can further alter gut tissues in response to a local immune sensitization to soybean protein components. The level of intake of digestible protein and energy was kept constant across the dietary treatments tested in order to exclude the influence of these factors.

2. MATERIALS AND METHODS

2.1. Animals and diets

Twenty Holstein calves [11 males and 9 females; mean birth body weight: 44.4 ± (SEM) 4.6 kg] were fitted with a T-piece silicone cannula in the duodenum, approximately 10 cm prior to the pancreatic duct, at an age of 3 weeks (mean body weight of 48.8 ± 1.3 kg). They were reared in individual pens on straw throughout the experiment.

The control diet was based on SMP and whey powder (*table I*). Two experimental diets containing a mixture of SMP and soybean products [1:1, on a digestible crude protein (CP) basis] were formulated. The soybean products were heated soybean flour (HSF, Protisoya from Société Industrielle des Oléagineux, Bougival, France) and ethanol-treated soybean protein concentrate (SPC, Danproveal from Central Soya, Aarhus, Denmark). The HSF product was considered as antigenic because it contained 132 and 30 mg of immunoreactive glycinin and β-conglycinin per gram CP, respectively, as determined by an ELISA assay [37]. In contrast, neither of these immunoreactivities were detected in the SPC product, which was therefore considered hypo-antigenic. The soya-based diets were formulated to contain similar levels of digestible CP and energy (*table I*). In the control diet, the calculated level of digestible CP and energy was slightly higher (+7 and +5 %, respectively) than in the soya-based diets. The calculated amounts of lactose were 410, 447 and 299 $g \cdot kg^{-1}$ DM, in the SMP, SPC and HSF diets, respectively.

2.2. Experimental procedure

The calves were initially fed the SMP diet for 2 weeks. Then they were randomly distributed into two groups that were fed either the HSF or the SPC diet [$n = 12$ (7 males and 5 females) and $n = 8$ (4 males and 4 females), respectively]. The calves were fed the reconstituted milk replacers twice daily at 08.30 and 16.30 hours using an open bucket at a level of 55 g DM kg^{-1} $W^{0.75}$. This represented approximately 90 % of the usual feed intake [18]. The calves were weighed weekly in order to adjust the amounts of distributed feed. Biopsy specimens were removed from the proximal jejunum just before (week 0), during (ends of weeks 2 and 8) and 2 weeks after (end of week 10) feeding of the soya-based diets. An adult-size Watson capsule (T.C. Components Ltd, Hampton, UK) [4] bonded to a PVC tubing (6 mm internal diameter) was inserted in the duodenum through the cannula and gently pushed through the gut. The mucosa was sampled approximately 1 m distally from the site of cannulation by suction using a 20-mL syringe. In each case, two specimens were collected, washed in physiological saline and observed under a dissecting microscope. One biopsy was fixed in phosphate-buffered formalin (10 %, pH 7.6) while the other was immediately frozen in liquid nitrogen and stored at –80 °C for subsequent analysis of enzyme activities.

2.3. Plasma anti-soya antibody titres

Plasma anti-soya antibody titres were determined with the passive haemagglutination test

Table I. Ingredient and chemical compositions of the experimental milk replacer diets.

	SMP	HSF	SPC
Diet ingredients ($g \cdot kg^{-1}$)			
Skimmed milk powder	540	270	270
Whey powder	170.0	74.4	74.4
HSF product		320.0	
SPC product			164.2
Fat[1]	210.0	225.6	180.0
Sucrose			23.8
Lactose		58.5	223.4
Raw starch	60		
Dicalcium phosphate		8.7	16.1
Sodium chloride		5.3	5.6
Potassium chloride			7.0
Calcium chloride		3.7	
Magnesium sulphate			1.7
Others	20.0[2]	33.8[3]	33.8[3]
Chemical composition ($g \cdot kg^{-1}$ DM)			
OM	926	929	926
Ash	74.3	71.0	74.4
CP (N × 6.25)	226	278	230
Fat	220	246	191
Digestible CP[4]	212	202	201
Digestible energy[4, 5]	4.96	4.67	4.58

[1] Mixture of tallow and coconut oil (81:19) in the SMP diet, and pure tallow in the HSF and SPC diets.
[2] Composition not disclosed by the manufacturer.
[3] L-lysine HCl 3.9, DL-methionine 2.0, pregelatinized starch 20.0, sorbitol 5.0, vitamin and mineral premix 2.9.
[4] Calculated from previous experiments with similar products in this laboratory.
[5] $Mcal \cdot kg^{-1}$ DM for digestible energy.

of sheep red blood cells using a saline extract of HSF ($1\ mg \cdot mL^{-1}$) as the coating antigen mixture [17]. Titres were defined as the number of doubling dilutions from the initial plasma dilutions of 1:20 which led to the disappearance of haemagglutination.

2.4. Histomorphometry

Methods for assessing the intestinal tissue morphology were based on those described by Goodlad et al. [6]. The specimen fixed in buffered formalin was transferred to a mixture (1:3) of acetic acid and ethanol for at least 24 h. It was stained with Schiff's reagent after hydrolysis in 1 N HCl at 60 °C for 6 min. Bands of villus-crypt units were cut, and individual crypts were further isolated from the connective tissue, using a fine-gauge syringe needle under a dissecting microscope. The preparation was mounted on a glass slide in a drop of 45 % acetic acid. Mitotic figures were counted by scanning through crypts under a microscope. Crypt depth, crypt width, villus height and villus width were measured using an image analyser (Seescan Imaging, Cambridge, UK). Mean values of these parameters were determined for at least ten individual crypts and villi from each calf at each sampling time.

2.5. Enzyme activities

The frozen biopsies were homogenized in ice-cold physiological saline using an ultrasonic probe (Microson™, Misonix Inc., NY, USA), fractionated in 100 µL aliquots and refrozen at –40 °C until analysis within 2 weeks.

The total activity of alkaline phosphatase (EC 3.1.3.1) was determined according to Babson and Read [1] using the following modifications [22]. 2-Amino-2-methyl propan-1-ol (0.25 M, pH 10.4; $MgCl_2$ 5 mM) and α-naphtyl phosphate (4 mM) were used as the buffer and substrate, respectively. Tissue homogenates were incubated

at 30 °C for 30 min. The reactions were stopped by adding sodium citrate 0.1 M, pH 5.2. A diazoic reaction was performed with o-dianisidine tetrazotized (30 mM) for 3 min at room temperature and stopped with 5 % trichloracetic acid. The coloured product of the diazoic reaction was extracted with ethyl acetate and the absorbance was measured at 530 nm using α-naphtol as the standard.

Lactase-phlorizin hydrolase (EC 3.2.1.23) activity was estimated according to Tivey et al. [34] by incubating aliquots of intestinal biopsy homogenates at 37 °C for 60 min with 50 mM lactose in 0.1 M sodium citrate buffer pH 6.0 containing 0.1 mM p-chloro-mercuri-benzoate to inhibit lysosomal β-galactosidase. Released glucose was determined using the glucose oxidase-peroxidase method (GOD-Périd kit no. 124 036, Boehringer Mannheim, GmbH Diagnostica, Mannheim, Germany). Absorbance of the reaction product in the samples and glucose standards was read at 610 nm.

The activities of amino-peptidase A (EC 3.4.11.7), amino-peptidase N (EC 3.4.11.2) and dipeptidyl-peptidase IV (EC 3.4.14.5) were determined by incubating biopsy homogenates at 37 °C for 30 min in 50 mM Tris buffer with a pH of 8.0, 7.3 and 8.0, respectively [28]. The substrates used were 1 mM α-L-glutamic acid 4-nitroanilide, 1 mM L-alanine 4-nitroanilide, and 1.5 mM glycyl-L-proline 4-nitroanilide, respectively [31]. The absorbance of the released p-nitroaniline was read at 410 nm using p-nitroaniline standards.

All the reagents used for determining enzyme activities were from Sigma Chemicals (Saint-Louis, MO, USA). The absorbances of all the reaction products were read on a Beckman DU-64 spectrophotometer (Beckman Instruments Inc., Fullerton CA, USA). Enzyme activities were related to the amount of protein present in tissue homogenates (μM hydrolysed substrate·mg^{-1} tissue protein·h^{-1}). Protein was determined using the Bio-Rad protein assay reagent (Bio-Rad, Hemel Hempstead, UK).

2.6. Statistical analysis

Data were analysed as repeated measures designed to test the effects of time, soybean antigenicity and antigenicity × time interaction using the repeated statement of the General Linear Models procedure of SAS [29]. Time was the whole plot, and each calf was a block in the subplot. Significant time effects were partitioned into single degrees of freedom orthogonal comparisons for linear, quadratic and cubic effects. When the treatments were significant, differences between means were separated using the Bonferroni test [29]. Differences were declared significant at $P < 0.05$ unless otherwise indicated.

3. RESULTS

3.1. Animal performance and plasma anti-soya antibody titres

The body weight gain of calves during the 12 weeks of the experiment was not significantly different ($P > 0.05$) between the HSF and the SPC groups (48.3 ± 1.2 and 50.3 ± 2.3 kg, respectively). The body weight gain for all the calves was 680 ± 59, 534 ± 53 and 689 ± 64 g/d during the periods of feeding SMP, soya-based diets and SMP, respectively. It was significantly higher for the periods based on SMP than during feeding of the soya-based diets ($P < 0.05$). The consumption of the SMP diet during the first 2 weeks (14.5 ± 0.5 and 15.1 ± 0.7 kg of powder), the soya-based diets during the following 8 weeks (78.0 ± 3.1 and 76.7 ± 3.0 kg of the HSF and SPC diets, respectively) and the SMP diet during the last 2 weeks (23.5 ± 0.7 and 24.2 ± 1.0 kg) were not significantly different ($P > 0.05$) between the experimental groups.

The plasma anti-soya antibody titres determined the week before the start of feeding of the soya-based diets were low and not significantly different ($P > 0.05$) between the HSF and SPC groups (0.1 ± 0.04 and 0.4 ± 0.4, respectively). After 8 weeks of soya consumption, the antibody titres were significantly higher ($P < 0.05$) in the HSF group as compared to the SPC group (4.7 ± 2.6 and 1.7 ± 1.3, respectively).

3.2. Jejunal morphology

The effects of antigenicity (i.e. HSF versus SPC) and antigenicity × time interaction were not significant ($P > 0.05$) for villus height, villus width, crypt depth, crypt

width and number of mitoses per crypt.

On the contrary, villus height decreased by 22 % ($P < 0.01$) after 2 weeks of soya consumption, a change that was restored 2 weeks after the calves had been returned to the SMP diet (*table II*). This related to the nature of dietary protein (SMP versus soya and vice-versa) rather than to an effect of time because feeding soya for 8 weeks, as compared to 2 weeks, did not significantly ($P > 0.05$) affect villus height. Contrasting with this, villus width increased linearly with time ($P < 0.0001$). It increased 28 % ($P < 0.001$) between weeks 2 and 8 of soya feeding. It did not change significantly ($P > 0.05$) after the switch from SMP to soya, and it only increased by 12 % ($P < 0.05$) 2 weeks after the calves were returned to SMP.

Crypt depth increased significantly ($P < 0.001$) in response to switching from SMP to soya protein, although the variations were not significant ($P > 0.05$) for the transition from soya to SMP. Crypt depth also tended ($P = 0.08$) to increase with time during the period of soya feeding. Crypt width also increased linearly with time ($P < 0.01$), an effect that was mainly observed soon after the switch from SMP to soya. The number of mitoses per crypt varied quadratically over time ($P < 0.05$). The only significant change, however, was its decrease ($P < 0.05$) when the calves were switched from soya to SMP at the end of the trial.

3.3. Enzyme activities of the jejunal mucosa

The effects of antigenicity and antigenicity × time interaction were never significant ($P > 0.05$) regardless of the specific enzyme activity measured.

In contrast, total alkaline phosphatase activity varied quadratically ($P < 0.0001$) over time (*table III*). It decreased by 9 % ($P < 0.01$) 2 weeks after SMP had been replaced by the soya-based diets, but increased by 82 % ($P < 0.0001$) when the calves were returned to the SMP diet. No significant change ($P > 0.05$) was observed in alkaline phosphatase activity after 8 weeks, as compared to 2 weeks, of soya feeding.

The lactase-specific activity tended to decrease ($P = 0.10$) 2 weeks after the start of the soya feeding, but it increased significantly (+60 %, $P < 0.01$) when the calves were returned to SMP. A significant decrease with time was observed between weeks 2 and 8 of soya feeding (–31 %, $P < 0.01$).

Changes in amino-peptidase A-specific activity tended to vary quadratically over time ($P = 0.06$), but time-to-time variations were not significant ($P > 0.05$).

The specific activity of amino-peptidase N varied linearly ($P < 0.001$) and quadratically ($P < 0.01$) over time. This was due to a significant decrease (–15 %, $P < 0.05$) in its activity 2 weeks after the calves started to consume the soya-based diets. A further decrease (–29 %, $P < 0.01$) was recorded between weeks 2 and 8 of soya feeding.

The specific activity of dipeptidyl-peptidase IV varied quadratically ($P < 0.0001$) over time. Changes in activity of that enzyme after 2 or 8 weeks of feeding soya were not significant ($P > 0.05$), but it increased strongly (+103 %, $P < 0.0001$) after switching from soya to SMP.

4. DISCUSSION

Besides the ontogenetic development, various aspects of nutrition and hormonal status, as well as infectious and non-infectious inflammations are known to affect small intestinal morphology and function. Among these factors, the level of food intake is important to consider because of its confounding effect in many studies [26]. Here, we chose a moderate feeding plan because at higher levels calves tend to refuse soya-based diets, especially with insufficiently processed products such as HSF [15].

Table II. Influence of protein source and antigenicity of soybean products on the morphology of the jejunal mucosa collected from preruminant calves successively fed milk replacer diets based on skimmed milk powder (SMP), whey and antigenic heated soybean flour (HSF) or hypo-antigenic soya protein concentrate (SPC), and skimmed milk powder again (means ± SEM).

	Time (weeks)[1]				Statistical analysis[2]			
Protein	0 SMP	2 Soya	8 Soya	10 SMP	Overall time effect	Week 0 versus week 2	Week 2 versus week 8	Week 8 versus week 10
Villus length (μm)					L ns, Q***, C ns	**	ns	*
HSF[3]	782 (34)	640 (52)	730 (58)	830 (48)				
SPC[3]	820 (67)	614 (59)	684 (57)	833 (66)				
Villus width (μm)					L****, Q*, C ns	ns	***	*
HSF	419 (37)	433 (31)	577 (49)	614 (38)				
SPC	445 (39)	418 (34)	509 (29)	605 (49)				
Crypt depth (μm)					L****, Q****, C ns	***	#	ns
HSF	322 (23)	419 (16)	444 (19)	416 (16)				
SPC	354 (27)	391 (20)	447 (40)	432 (22)				
Crypt width (μm)					L**, Q #, C ns	**	ns	ns
HSF	114 (6)	137 (6)	146 (9)	137 (9)				
SPC	116 (9)	123 (6)	134 (11)	138 (9)				
Mitoses per crypt					L ns, Q #, C ns	ns	ns	*
HSF	3.7 (0.3)	4.8 (0.4)	4.8 (0.6)	3.6 (0.6)				
SPC	4.1 (0.9)	4.3 (0.7)	5.4 (1.1)	3.4 (0.5)				

[1] Biopsies collected just before (week 0), during (ends of weeks 2 and 8) and 2 weeks after (end of week 10) feeding of the soya-based diets.
[2] Antigenicity (i.e. HSF versus SPC) and antigenicity × time interaction were never significant ($P > 0.05$).
Linear (L), quadratic (Q) or cubic (C) effect of time and differences between successive measurements (week 0 versus week 2, week 2 versus week 8 and week 8 versus week 10). # $P < 0.10$, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
[3] HSF diet based on antigenic heated soybean flour, SPC diet based on hypo-antigenic soya protein concentrate.

Table III. Influence of protein source and antigenicity of soybean products on the specific activity of enzymes (μM substrate degraded/mg tissue protein·h^{-1}) of the jejunal mucosal collected from pre-ruminant calves successively fed milk replacer diets based on skim milk powder (SMP), whey and antigenic heated soybean flour (HSF) or hypo-antigenic soya protein concentrate (SPC) and skimmed milk powder again (means ± SEM).

Time (weeks)[1]	Statistical analysis[2]							
Protein	0 SMP	2 Soya	8 Soya	10 SMP	Overall time effect	Week 0 versus week 2	Week 2 versus week 8	Week 8 versus week 10
Total alkaline phosphatase					L ns, Q ****, C ns	**	ns	****
HSF[3]	32.0 (4.4)	18.5 (3.4)	18.0 (3.3)	35.5 (2.6)				
SPC[3]	31.2 (6.0)	20.1 (6.9)	17.3 (3.7)	29.0 (3.4)				
Lactase					L *, Q **, C #	#	**	**
HSF	3.95 (0.39)	3.50 (0.46)	2.19 (0.26)	3.93 (0.32)				
SPC	4.45 (0.66)	2.87 (0.41)	2.43 (0.31)	3.46 (0.57)				
Amino-peptidase A					L ns, Q #, C ns	ns	ns	ns
HSF	82.2 (4.4)	114.6 (12.3)	83.6 (17.9)	91.0 (12.3)				
SPC	90.0 (24.2)	102.2 (20.2)	118.3 (16.1)	84.5 (10.0)				
Amino-peptidase N					L ***, Q **, C ns	*	**	ns
HSF	72.3 (7.4)	64.8 (6.1)	49.4 (8.9)	51.1 (5.8)				
SPC	84.5 (8.2)	67.7 (7.9)	45.1 (3.7)	54.0 (4.6)				
Dipeptidyl-peptidase IV					L *, Q ****, C *	ns	ns	****
HSF	409 (88)	361 (86)	330 (88)	636 (101)				
SPC	420 (71)	309 (49)	360 (58)	763 (127)				

[1] Biopsies collected just before (week 0), during (ends of weeks 2 and 8) and 2 weeks after (end of week 10) feeding of the soya-based diets.
[2] Antigenicity (i.e. HSF versus SPC) and antigenicity by time interaction were never significant ($P > 0.05$).
Linear (L), quadratic (Q) or cubic (C) effect of time and differences between successive measurements (week 0 versus week 2, week 2 versus week 8 and week 8 versus week 10). # $P < 0.10$, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.
[3] HSF diet based on antigenic heated soybean flour, SPC diet based on hypo-antigenic soya protein concentrate.

4.1. Lack of effects of soya antigenicity

The present experiment was designed to further analyse the impact of dietary antigens on the small intestinal mucosa, based on our previous work on the allergenicity of soya in calves [16, 17]. The immunogenicity of the HSF product tested here was rather moderate because anti-soya antibody titres in plasma were 3 units (i.e. 8-fold) lower than in our previous studies, after similar periods of soya feeding. This, together with the lack of diarrhoea and effects of soya antigenicity on the small intestine morphology, epithelial proliferation and enzyme activities, suggest that the calves were fairly tolerant to HSF. It must be kept in mind, however, that in sensitive calves, immune-mediated gut disorders to HSF are associated with partial villus atrophy and crypt hyperplasia [14, 25]. In this situation, not observed here, changes in villus height and crypt depth may be linked with underlying cellular events, among which the activation of local T lymphocytes could play an important role [3, 33]. Although major changes in immune cell populations of the mucosa were not observed here (J.P. Lallès et al., unpublished data), the hypothesis of the immune modulation of intestinal morphology and dynamics is consistent with our previous observations that the density of T lymphocytes was increased in the mucosa of sensitive calves [16]. Nevertheless, switching calves from SMP to soya negatively affected the jejunal villus height and enzyme activities, probably reflecting an increased immaturity of enterocytes along the villus axis. This may favour sensitization in predisposed animals because Heyman et al. [10] demonstrated a higher antigen absorption in immature cells.

4.2. Influence of the nature of dietary protein and other factors

One major result obtained in this study was that villus height and specific activities for most enzymes studied decreased in response to switching from SMP to soya, an effect that was largely reversed 2 weeks after SMP refeeding. This is in agreement with previous observations made by Grant et al. [7] who found that jejunal villus height and lactase activity were reduced in calves fed SPC. Epithelial cell proliferation tended to increase, however, with soya feeding, while it was shown to decrease in the work by Grant et al. [7]. In that study, the incorporation rate of soybean protein and the levels of feeding were lower than in the present experiment. Nevertheless, additional factors other than the nature of protein per se may also be involved in the changes observed in intestinal morphology and enzyme activities.

Villus height has been shown to correlate positively with daily body weight gain in growing rats [41]. Here, the daily body weight was lower during soya feeding than during both SMP periods. We did not find, however, any significant relationship between body weight gain and villus height.

The major differences between our control diet based on SMP and those containing soya products, in addition to the nature of the protein, were the clotting ability and levels of lactose and carbohydrates. Milk replacers containing substantial amounts of soya products do not clot, contrary to SMP diets [35]. This increases the rate of abomasal emptying of protein and fat, possibly shifting the site of digestion distally, with consequences on luminal nutrition of the intestinal mucosa. To our knowledge, the impact of changes in the clotting ability of milk replacers on the morphology and function of the small intestine has not yet been investigated.

Small intestinal morphology and enzyme activities were significantly modified after dietary switches from SMP to both the HSF and SPC diets, and vice-versa (*tables II* and *III*). High doses of lactose stimulate lactase activity in calves [11]. The changes in lactase activity observed here are probably not due to changes in lactose concentrations in

the formulas because the HSF diet contained approximately 30 % less lactose than the SMP or the SPC diets.

Decreasing the concentration of protein in isoenergetic diets and starvation negatively affect villus height, crypt depth and/or crypt cell proliferation in the small intestines of rats and pigs [5, 24, 41]. On the contrary, the effects of the nature of dietary protein on intestinal morphology and function have rarely been studied. Seegraber and Morrill [30] noted that calves fed casein had villi which were less uniform than those fed SMP after 6 weeks, and became shorter and broader after 10 weeks. Protein intake was, however, also lower with casein. Blunting and shortening of villi were recorded after 4 weeks of soya feeding [30]. But this observation was evidenced even after only a few days, and interpreted as a direct toxic effect of soya on the mucosa [14]. Seegraber and Morrill [30] also observed abnormal intestinal villi, together with increased diarrhoea and mortality, by feeding a fish protein concentrate to calves. As an explanation, they implicated possible deficiencies in certain essential amino acids of fish proteins. In most studies on calves, the intestinal mucosa returned to normal within 2 weeks of feeding a milk replacer diet based on SMP [14, 30]. We confirmed the reversibility of this phenomenon here, even in the absence of severe immune responses.

Other dietary factors have been documented to alter the dynamics of small intestinal mucosa. For example, the spillage of large amounts of the milk replacer in the forestomach, probably involving an insufficient closure of the reticular groove, causes the 'ruminal drinking' syndrome in calves [38, 39]. This is associated with villus atrophy of the jejunum and the reduced activities of alkaline phosphatase and lactase. Although the possible link between intestinal villous atrophy and the fermentation of milk components in the rumen has not been elucidated thus far, part of the effect may arise from the low feed intake in these calves. This syndrome may be amplified by substituting SMP with non-clotting protein such as soya.

The dynamics of the intestinal morphology and enzyme activity are modulated by many biological substances including hormones (glucocorticoids [28]; tri-iodo-thyronine [34]; insulin [2]), growth factors (EGF, IGF, TGF [27]) and polyamines [13]. The influence of most of these factors has not yet been studied in the calf. However, polyamine supplementation was shown to reverse partial jejunal villus atrophy and to reduce epithelial cell proliferation observed in calves fed SPC [7]. Gastrin and perhaps CCK are important for mucosal growth of the small intestines but the actual mechanisms are not completely understood [13]. Le Dréan et al. [19] recently observed that plasma concentrations of gastrin and CCK, expressed as a proportion of their preprandial values, showed a higher post-prandial increase in calves fed a milk replacer diet containing SPC than in those fed SMP. Whether these observations are relevant to explain the present differences in intestinal morphology and function is unknown.

Data relating the impact of the nature of proteins to intestinal enzyme activities are scarce. In rats, sucrase activity was found to be lower with zein or gelatin than with gluten or casein, probably because the former sources are very deficient in most amino acids [12]. Alkaline phosphatase activity was also lower with lactalbumin, egg albumin, zein, gelatin or wheat gluten than with the phosphoproteins casein and vitellin [36]. Feeding a high-proline-rich diet resulted in an increased expression of intestinal brush border dipeptidyl peptidase IV [32]. Soya protein contains about 40 % less proline than milk protein [8]. This could have partially contributed to the variations observed here for the activity of this enzyme.

Among dietary factors, increasing the protein level in the diet from 5–6 to 25–26 % increased the mucosal activities of alkaline phosphatase and amino-peptidase N, but decreased that of lactase [9, 40]. Dietary restriction was shown to increase lactase

and leucine amino-peptidase activities in the proximal intestine of nursing piglets [24].

4.3. Effects of age and/or long-term effects of soya

In the study by Le Huërou et al. [20], after the first weeks of life in preruminant calves fed a milk replacer based on SMP, the specific activity of lactase in the entire small intestine does not vary significantly with age, whereas in the case of amino-peptidase N it increases. Here we observed a general reduction of lactase and amino-peptidase N activities, together with an enlargement of villi, in the proximal jejunum. These apparent discrepancies may be due to regional variations in the regulation of enzyme activities [20] and possibly due to differential interactions with the dietary proteins used.

5. CONCLUSION

Feeding milk replacers containing antigenic or hypo-antigenic soya protein to calves reversibly depressed villus height and the specific activity of a number of brush border enzymes of the proximal jejunum, except for amino-peptidase A. Whether this may explain the lower digestibility usually observed with heated soybean flours or concentrates is still uncertain. Factors other than the nature of the protein per se (e.g. abomasal emptying rate and carbohydrates) may also alter intestinal morphology and function, and therefore warrant further investigation.

ACKNOWLEDGEMENTS

The authors thank all of the animal facility staff for calf care and Mrs J. Quillet for gathering the literature.

REFERENCES

[1] Babson A.L., Read P.A., A new assay for prostatic acid phosphatase in serum, Am. J. Clin. Pathol. 32 (1959) 88–91.

[2] Buts J.P., de Keyser N., Kolanowski J., van Hoof F., Hormonal regulation of the rat small intestine: responsiveness of villus and crypt cells to insulin during the suckling period and unresponsiveness after weaning, Pediatr. Res. 27 (1990) 161–164.

[3] Cunha Ferreira R. da, Forsyth L.E., Richman P.I., Wells C., Spencer J., MacDonald T.T., Changes in the rate of crypt epithelial cell proliferation and mucosal morphology induced by a T-cell mediated response in human small intestine, Gastroenterology 98 (1990) 1255–1263.

[4] Evans N., Farrow L.J., Harding A., Stewart J.S., New techniques for speeding small intestinal biopsies, Gut 11 (1970) 88–89.

[5] Goodlad R.A., Plumb J.A., Wright N.A., Epithelial cell proliferation and intestinal absorptive function during starvation and refeeding in the rat, Clin. Sci. 74 (1988) 301–306.

[6] Goodlad R.A., Levi S., Lee C.Y., Maudir N., Hodgson H., Wright N.A., Morphometry and cell proliferation in endoscopic biopsies: evaluation of a technique, Gastroenterology 101 (1991) 1235–1241.

[7] Grant A.L., Holland R.E., Thomas J.W., King K.J., Liesman, J.S., Effects of dietary amines on the small intestine in calves fed soybean protein, J. Nutr. 119 (1989) 1034–1041.

[8] Guilloteau P., Toullec R., Grongnet J.F., Patureau-Mirand P., Prugnaud J., Sauvant D., Digestion of milk, fish and soya-bean protein in the preruminant calf: flow of digesta, apparent digestibility at the end of the ileum and amino acid composition of ileal digesta, Br. J. Nutr. 55 (1986) 571–592.

[9] Guiraldes E., Hamilton J.R., Effect of chronic malnutrition on intestinal structure, epithelial renewal, and enzymes in suckling rats, Pediatr. Res. 15 (1981) 930–934.

[10] Heyman M., Crain-Denoyelle A.M., Desjeux J.F., Endocytosis and processing of protein by isolated villus and crypt cells of the mouse small intestine, J. Pediatr. Gastroenterol. Nutr. 9 (1989) 238–245.

[11] Huber J.T., Rifkin R.J., Keith J.M., Effect of level of lactose upon lactase concentrations in the small intestines of young calves, J. Dairy Sci. 47 (1964) 789–797.

[12] Johnson A.O.K., The effects of dietary protein quality and carbohydrate quantity on the activity of intestinal dissacharidase in Wistar rats, Scand. J. Gastroenterol. 15 (1980) 219–223.

[13] Johnson L.R., McCormack S.A., Regulation of gastrointestinal mucosal growth, in: Johnson L.R. (Ed.), Physiology of the Gastrintestinal Tract, 4th ed., Raven Press, New York, USA, 1984, pp. 611–641.

[14] Kilshaw P.J., Slade H., Villus atrophy and crypt elongation in the small intestine of preruminant calves fed with heated soya bean flour or wheat gluten, Res. Vet. Sci. 33 (1982) 305–308.

[15] Lallès J.P., Nutritional and antinutritional aspects of soyabean and field pea proteins used in veal calf production: a review, Livest. Prod. Sci. 34 (1993) 181–202.

[16] Lallès J.P., Dréau D., Féménia F., Parodi A.L., Toullec R., Feeding heated soyabean flour increases the density of B and T lymphocytes in the small intestine of preruminant calves, Vet. Immunol. Immunopathol. 52 (1996) 105–115.

[17] Lallès J.P., Dréau D., Salmon H., Toullec R., Identification of soya bean allergens and immune mechanisms of dietary sensitivities in preruminant calves, Res. Vet. Sci. 60 (1996) 111–116.

[18] Lallès J.P., Tukur H.M., Toullec R., Miller B.G., Analytical criteria for predicting apparent digestibility of soybean protein in preruminant calves, J. Dairy Sci. 79 (1996) 475–484.

[19] Le Dréan G., Le Huërou-Luron I., Gestin M., Romé V., Plodari M., Bernard C., Chayvialle J.A., Guilloteau P., Comparison of the kinetics of pancreatic secretion and gut regulatory peptides in the plasma of preruminant calves fed milk or soybean protein, J. Dairy Sci. 81 (1998) 1313–1321.

[20] Le Huërou I., Guilloteau P., Wicker C., Mouats A., Chayvialle J.A., Bernard C., Burton J., Toullec R., Puigserver A., Activity distribution of seven digestive enzymes along small intestine in calves during development and weaning, Dig. Dis. Sci. 37 (1992) 40–46.

[21] Leterme P., Monmart T., Théwis A., Morandi P., Effect of oral and parenteral N nutrition vs N-free nutrition on the endogenous amino acid flow at the ileum of the pig, J. Sci. Food Agric. 71 (1996) 265–271.

[22] Martins M.J., Dias P.O., Alçada M.N.M.P., Hipolito-Reis C., Evaluation of serum alkaline phosphatase activity using different assay methods, 17th International Congress on Biochemistry and Molecular Biology, San Francisco, USA, abstract, 1997.

[23] Nielsen N.C., Deshpande S.S., Hermodson M.A., Scott M.P., Comparative digestibility of legume storage proteins, J. Agric. Food Chem. 36 (1988) 896–902.

[24] Nunez M.C., Bueno J.D., Ayudarte M.V., Allmendros A., Rios A., Suarez M.D., Gil A., Dietary restriction induces biochemical and morphological changes in the small intestine of nursing piglets, J. Nutr. 126 (1996) 933–944.

[25] Pedersen H.E., Sissons J.W., Effect of antigenic soyabean protein on the physiology and morphology of the gut in the preruminant calf, Can. J. Anim. Sci. 64 (suppl.) (1984) 183–184.

[26] Pluske J.R., Hampson D.J., Williams I.H., Factors influencing the structure and function of the small intestine in the weaned pig: a review, Livest. Prod. Sci. 51 (1997) 215–236.

[27] Potten C.S., Owen G., Hewitt D., Chadwick C.A., Hendry H., Lord B.I., Woolford L.B., Stimulation and inhibition of proliferation in the small intestinal crypts of the mouse after in vivo administration of growth factors, Gut 36 (1995) 864–873.

[28] Sangild P.T., Sjöström H., Noren O., Fowden A.C., Silver M., The prenatal development and glucocorticoid control of brush-border hydrolases in the pig small intestine, Pediatr. Res. 37 (1995) 207–212.

[29] SAS/STAT®, User's Guide, Version 6, Fourth Edition, Volume 2, SAS Inst. Inc., Cary, NC, USA, 1990.

[30] Seegraber F.J., Morrill J.L., Effect of protein source in calf milk replacers on morphology and absorptive ability of the small intestine, J. Dairy Sci. 69 (1986) 460–469.

[31] Sjöström H., Noren O., Jeppesen L., Staun M, Svensson B., Christiansen L., Purification of different amphiphilic forms of a microvillus aminopeptidase from pig small intestine using immunoadsorbent chromatography, Eur. J. Biochem. 88 (1978) 503–511.

[32] Suzuki Y., Erickson R.H., Sedlmayer A., Chang S.K., Ikihara Y., Kim Y.S., Dietary regulation of rat intestinal angiotensin-converting enzyme and dipeptidyl peptidase IV, Am. J. Physiol. 264 (1993) 1153–1159.

[33] Thompson F.M., Mayrhofer G., Cummins A.G., Dependence of epithelial growth of the small intestine on T-cell activation during weaning in the rat, Gastroenterology 111 (1996) 37–44.

[34] Tivey D.R., Morovat A., Dauncey M.J., Administration of 3,5,5'-triiodothyronine induces a rapid increase in enterocyte lactase-phlorizinn hydrolase activity of young pigs on a low energy intake, Exp. Physiol. 78 (1993) 337–346.

[35] Toullec R., Lallès J.P., La digestion dans la caillette et l'intestin grêle, in: Jarrige R., Ruckebusch Y., Demarquilly C., Farce M.H., Journet M. (Eds.), Nutrition des ruminants domestiques: ingestion et digestion, Inra Publications, Paris, 1995, pp. 527–582.

[36] Tuba J., Dickie N., The role of alkaline phosphatase in intestinal absorption. IV- The effects of various proteins on levels of the enzyme in intestinal mucosa, Can. J. Biochem. Physiol. 33 (1955) 89–92.

[37] Tukur H.M., Lallès J.P., Mathis C., Caugant I., Toullec R., Digestion of soybean globulins glycinin, α-conglycinin and β-conglycinin, in the preruminant and the ruminant calf, Can. J. Anim. Sci. 73 (1993) 891–905.

[38] van Weeren-Keverling Buisman A., Noordhuizen-Stassen E.N., Breukink H.J., Wensing T., Mouwen J.M.V.M., Villus atrophy in ruminal drinking calves and mucosal restoration after reconditioning, Vet. Q. 10 (1988) 164–171.

[39] van Weeren-Keverling Buisman A., Mouwen J.M.V.M., Wensing T., Breukink H.J., Intraluminal administration of milk in the calf as a model for ruminal drinking: morphological and enzymatic changes in the jejunal mucosa, Vet. Res. Commun. 14 (1990) 129–140.

[40] Zambonino-Infante J.L., Rouanet J.M., Caporiccio B., Besançon P., Effects of dietary protein and carbohydrate level in the rat small intestine: enzymic, histological and electron microscopy studies, Nutr. Rep. Int. 40 (1989) 313–321.

[41] Zambonino-Infante J.L., Rouanet J.M., Besançon P., Mathematical correlation between villus height and the nutritional state in Sprague-Dawley rats, Gut 34 (1993) 1066–1068.