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Original article

Digestibility, blood levels of nutrients and skin responses of calves fed soyabean and lupin proteins

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Summary — Three milk substitute diets in which the protein was provided either by skim milk only (control diet) or mainly (71%) by a commercial soyabean or lupin concentrate (soyabean or lupin diet, respectively) were given to intact or ileo-caecal-cannulated preruminant calves. *In vitro* tests showed that both concentrates were partially proteolysed and had low antigenic and antitryptic activities. The low antigenicity was confirmed *in vivo* since none of the calves produced specific plasma antibodies against dietary proteins, and skin reactions following the injection of these proteins were minor. Postprandial plasma level of triglycerides was higher with the 2 legume diets, suggesting faster abomasal emptying of fat and probably protein. Apparent faecal nitrogen digestibility was lower ($P \leq 0.05$) with the soyabean and lupin diets than with the control diet (0.86, 0.88 and 0.95, respectively). At the ileal level, the differences were smaller and non-significant (0.90, 0.88 and 0.92) for nitrogen, but remained significant for valine and tyrosine with the soyabean diet, and for proline, valine, methionine, leucine and lysine with the lupin diet. However, the differences were small enough to conclude that proper denaturation of soyabean and lupin proteins by processes including partial hydrolysis can suppress their antigenicity and render them very digestible.

digestion / skin response / preruminant calf / soyabean / lupin

Résumé — Utilisation des protéines de soja et de lupin chez le veau préruminant : digestibilité, taux plasmatique des nutriments et réponses cutanées. *Trois laits de remplacement dans lesquels les protéines étaient apportées en totalité par de la poudre de lait écrémé (régime témoin) ou en majeure partie (71%) par un concentrat commercial de soja ou de lupin (régime soja et lupin respectivement) ont été distribués à des veaux préruminants intacts ou munis d'une canule réentrante iléocæcale. Les protéines des 2 concentrats avaient été partiellement hydrolysées et leurs activités antigénique et antitrypsique étaient très faibles* in vitro. *Il en a été de même* in vivo *pour l'activité antigénique puisque les veaux n'ont pas produit d'anticorps ni développé de réactions cutanées à la suite de l'in-*

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jection des protéines des concentrats. Les taux plasmatiques de triglycérides ont été plus élevés après le repas avec les aliments soja et lupin, suggérant une accélération de l'évacuation gastrique des lipides et probablement des protéines. La digestibilité apparente de l'azote a été moins élevée (P ≤ 0,05) au niveau fécal avec les régimes soja et lupin qu'avec le régime témoin (respectivement 0,86, 0,88 et 0,95). À la fin de l'iléon, les différences ont été moins fortes et non significatives (0,90, 0,88 et 0,92) pour l'azote, mais elles sont restées significatives pour la valine et la tyrosine avec le régime soja, et pour la proline, la valine, la méthionine, la leucine et la lysine avec le régime lupin. Cependant, les différences par rapport au régime témoin sont demeurées faibles. En conclusion, la dénaturation des protéines de soja et de lupin par des traitements entraînant à leur hydrolyse partielle permet de supprimer leur antigénicité et de les rendre très digestibles.

digestion / réponses cutanées / veau préruminant / soja / lupin

INTRODUCTION

Inclusion of legume proteins in the diets of preruminant calves has been associated with reduced animal performance. This is partly due to the rapid gastric emptying of legume-based diets and their resistance to digestive enzymes (Jenkins *et al,* 1980; van Kempen and Huisman, 1991), which lead to decreased nutrient digestibility (Guilloteau *et al,* 1986; Nunes do Prado *et al,* 1989a). Heated soyabean flour is the most widely used legume protein in monogastric feeding. However, it has been shown to cause immunological reactions in preruminant calves, which in severe cases can lead to alteration of the intestinal morphology of sensitized animals (Barratt *et al,* 1978; Kilshaw, 1981; Seegraber and Morrill, 1986). These adverse reactions can however be reduced by different processing methods, therefore allowing for higher inclusion levels. More recently, there has been a growing interest in the use of crops that can grow in European conditions (see review by Lallès, 1993). However, the utilization of some of these crops, such as pea, is limited by their low protein content and perhaps their high antigenic activities (Nunes do Prado *et al,* 1989b; Bush *et al,* 1992). Thus the exploration of other temperate-growing legumes, such as lupins, becomes of interest particularly because of their high content of protein in the seed, which compares favorably with that of soyabean, and in some species is highest among legumes (Cerletti, 1993). In addition, lupins contain lower levels of toxic and antinutritional factors compared to other legume seeds. For example, haemagglutinins and trypsin inhibitors are said to be absent, while alkaloids, which in wild varieties may reach 2.5% of dry seed weight, constitute only 0.01% in sweet varieties, thus rendering the flour acceptable for animal and even human consumption (Hudson, 1979). The level of flatulence factors (mainly oligosaccharides) is similar to those of soyabeans in *Lupinus albus* and *L angustifolius*, but are considerably higher in other varieties (Hudson, 1979).

This study was conducted to determine the effects of processed soyabean and lupin proteins on performance, ileal and faecal digestibility, and gastric emptying in preruminant calves. Levels of circulating antibodies as well as skin responses of calves to various dietary proteins were also evaluated.

MATERIALS AND METHODS

Diets

Two protein concentrates containing 59 and 43% crude protein (CP, % dry matter (DM)) were prepared from commercial defatted soyabean *(Glycine max)* and lupin (*L albus*, cultivar Lucky) flour, respectively (table I). According to the manufacturer (Bonilait Protéines, Chasseneuil-du-

Table I. Chemical composition of the protein sources and diets (% dry matter, ppm for trace minerals).

	Protein source		*Diet*		
	Soyabean	*Lupin*	*Control*	*Soyabean*	*Lupin*
Moisture	5.0	6.5	5.8	2.7	6.0
Crude protein [a]	58.6	43.1	21.5	21.1	21.5
Fat	6.2	7.2	21.4	21.2	20.4
N-free extract	28.0	45.4	50.1	50.1	50.6
Ash	7.2	4.3	7.0	7.6	7.5
Ca	0.36	0.22	1.20	1.1	1.17
P	0.66	0.48	0.79	0.88	0.95
K	0.72	0.51	1.06	0.94	0.93
Na	0.93	0.11	0.41	0.49	0.38
Mg	0.13	0.07	0.12	0.12	0.13
Cl	0.13	0.11	0.99	0.12	1.58
Fe	89	89	8	32	43
Zn	23	24	91	81	89
Mn	16	447	56	53	159
Cu	7	4	7	8	6

[a] N x 6.25.

Poitou, France), both concentrates were obtained by precipitation at pH 4.5 with hydrochloric acid from water suspension. This resulted in a 50–60% decrease in the contents of potassium, magnesium, zinc and manganese (table I) and an 80% decrease in soluble sugars (data not shown), compared to those found in defatted soyabean (Lallès *et al*, 1994) and lupin (Hudson, 1979; INRA, 1984) flours. In contrast, sodium was increased by 570 and 400%, while chloride was increased by 1500 and 150% for the soyabean and lupin, respectively, suggesting that sodium hydroxide was used to neutralise hydrochloric acid. The soyabean concentrate exhibited moderate glycinin immunoreactivity *in vitro*, equivalent to 1.05 g/100 g CP, which represents about 4 and 32% of the activities found in raw and heated soyabean flours, respectively (Lallès *et al*, 1993a). No immunoreactive β-conglycinin was detected. Electrophoretic band patterns of the 2 concentrates (fig 1) indicated at least a partial hydrolysis of proteins of high molecular weight. The results of antigenicity and electrophoresis strongly suggest that supplementary treatment not indicated by the manufacturer had been applied to both concentrates. The antitryptic activity was also very low: 1.4 and 1.2 trypsin units inhibited (TUI)/mg protein for the soyabean and lupin concentrates, respectively, compared to 4.7 TUI/mg protein for 2 commercial alcohol-treated soyabean concentrates, and 98 TUI/mg protein for a laboratory-defatted raw soyabean flour.

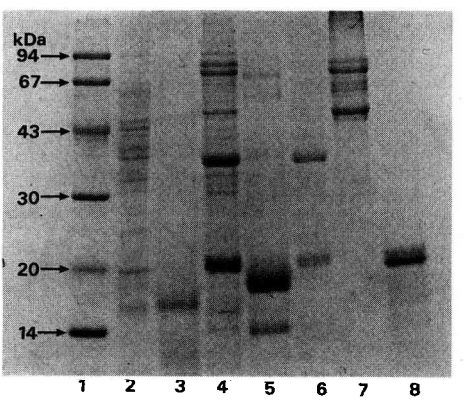

Fig 1. SDS-PAGE electrophoresis of MW standards (lane 1), raw lupin flour (lane 2), the lupin concentrate (lane 3), raw soyabean flour (lane 4), the soyabean concentrate (lane 5), and purified glycinin (lane 6), β-conglycinin (lane 7) and α-conglycinin (lane 8).

Three milk replacer diets (control, soyabean and lupin) containing approximately 21% CP and 21% fat (DM basis) were prepared. In the control diet, all the protein was provided by skim-milk powder (SMP) (table II). In the other 2 diets, 71% of the protein was from one of the 2 concentrates and the remaining was provided by whey proteins and synthetic amino acids (AA). L-Lysine-HCl and DL-methionine were added in order to obtain total levels of 1.8 and 0.9% lysine and sulfur amino acids, respectively. The AA composition of the protein concentrates and the diets is given in table III.

Experiment 1

Animals, feeding and digesta collection

Nine Holstein male calves were purchased at about 8 d of age, and fed skim-milk-based milk replacer from open buckets. At about 2 months of age, the animals started receiving the experimental diets. Three experimental periods were carried out successively. Each calf received each experimental diet for 2 weeks in different orders. Feeding was carried out by means of open buckets and was done twice daily (at 8.30 and 16.30 h). The amount of feed offered (58 g DM/d/kg$^{0.75}$) was adjusted weekly to animal body weight. The switch between 2 test diets was accomplished over 2 d as described by Bush *et al* (1992).

Faeces were collected totally for 5 d during the second week of each experimental period, and representative samples (from the 5-d collection period) were frozen for subsequent freeze-drying and analysis.

Table II. Composition of diets (%).

Ingredient	*Diet*		
	Control	*Soyabean*	*Lupin*
Fat premix 1 [a]	49.25	–	–
Fat premix 2 [b]	–	50.12	43.72
Skim-milk powder	30.46	–	–
Soyabean concentrate	–	26.07	–
Lupin concentrate	–	–	35.69
Whey powder	–	7.36	8.82
Whey concentrate	–	2.35	2.35
Lactose	15.5	9.38	3.77
Starch	2.0	–	–
L-Lysine-HCl	0.124	0.487	0.746
DL-Methionine	0.167	0.251	0.374
Vitamins and minerals	2.46	3.98	4.54

[a,b] Spray-dried fat premix containing 40% fat (99.7% tallow + 0.3% emulsifier) homogenized into 60% skim milk[a] or whey[b] powder.

Blood sampling

In order to evaluate gastric emptying by studying the time-course appearance of triglycerides and glucose, blood samples were taken before the morning meal (t_0) and then 1, 2, 3, 4 and 6.5 h after, during the fourth or fifth day of each experimental period. The samples were collected from the jugular vein into heparinized tubes, immediately centrifuged, and the plasma frozen until analysis. At the end of the 3 experimental periods, animals were allotted to 2 groups receiving either the soyabean or the lupin diet for an additional 2 months, during which blood samples were collected twice monthly. This was in order to study the possible development of circulating antibodies against the 2 legume proteins.

Skin tests

At the end of the trial, skin responses of the calves against different dietary protein extracts were analysed. The tests were carried out by intradermal injections of 200 µl of physiological saline solution containing 1 or 5 mg protein extracts of the soyabean concentrate, the lupin concentrate, a heated soyabean flour (HSF), a hydrolysed soyabean protein and SMP. Histamine (10^{-3} M) and physiological saline alone were also injected as positive and negative controls, respectively. Injections were carried out on the 2 shaved sides of the tranquilized (Calmivet, Laboratoire Vétroquil, Lure, France) calves, and the diameter of the oedema was measured at 1, 4 and 8 h post-injection in order to analyse short-term reactions. Long-term effects were evaluated by measuring the increase in double skin fold thickness (Heppell *et al*, 1987) at 24 and 48 h after injection. Control animals for this trial were taken from another batch of calves fed only SMP.

Table III. Amino-acid (AA) composition of protein sources and diets (g/100 g AA).

	Protein source		*Diet*		
	Soyabean concentrate	*Lupin concentrate*	*Control*	*Soyabean*	*Lupin*
ASP	12.01	11.50	7.78	11.55	10.93
THR	4.05	3.41	4.20	4.78	4.20
SER	5.56	5.46	5.41	5.35	5.21
GLU	19.51	24.05	21.89	18.91	21.88
PRO	5.42	4.34	9.45	5.26	4.85
GLY	4.15	3.76	1.85	3.49	3.25
ALA	4.29	3.10	3.06	4.34	3.46
CYS	1.72	1.47	0.84	1.84	1.66
VAL	5.04	4.57	6.46	5.17	4.68
MET	1.74	0.75	2.78	2.39	2.48
ILE	4.86	4.72	5.27	5.08	4.81
LEU	7.24	7.53	8.73	7.93	7.87
TYR	3.46	4.61	4.49	3.14	4.22
PHE	5.05	4.22	4.70	4.68	4.08
HIS	2.60	1.97	2.42	2.26	1.88
LYS	6.24	4.30	7.53	8.04	6.60
ARG	7.24	10.26	3.14	5.78	7.96
ΣAA (g/16 gN)	100.19	102.60	104.76	99.67	100.32
AAN (% total N)	85.17	88.53	82.33	83.25	84.75

N: nitrogen; ΣAA: sum of assayed AA; AAN: nitrogen of assayed AA except amide-N of asparagine and glutamine.

Experiment 2

The same experimental procedure (*ie* 3 diets fed during 3 different periods) was carried out with 5 Holstein heifer calves. At about 2.5 months of age, they were fitted with an abomasal catheter and a reentrant cannula whose proximal part was inserted at the distal ileum and the distal part in the caecum (Guilloteau *et al,* 1986). After calves had recovered from surgery, they were kept in metal frame stalls where they were fed the experimental diets. Diets were infused twice daily into the abomasum. The amount of feed offered was as in *Experiment 1.*

Ileal digesta were collected from reentrant cannulae over the last 4 d of the second week of each experimental period as detailed by Guilloteau *et al* (1986). Digesta were collected under continuous stirring in flasks containing sodium benzoate (10 g/kg digesta) to limit microbial activity. Digesta were weighed every day, and aliquots from the 4-d collection period were frozen for subsequent freeze-drying and analysis.

Chemical analyses

Diets and digesta samples were analysed for DM, nitrogen (N), fat and ash according to methods described previously (Guilloteau *et al,* 1986). The protein sources and ileal samples were also analysed for AA by ion exchange chromatography (Pharmacia-LKB analyser) after acid hydrolysis of samples in 6 N HCl at 110°C for 24 (48 h in the case of valine and isoleucine). For the determination of sulfur AA, oxidation with performic acid was carried out prior to acid hydrolysis (Prugnaud and Pion, 1976).

Glycinin and β-conglycinin immunoreactivities of the soyabean concentrate were assayed according to previously described methods (Tukur *et al,* 1993). The antitryptic activity of the legume concentrates was determined according to the AOCS methods (1983). SDS-PAGE electrophoresis (Laemmli, 1970) was carried out under reducing conditions for the 2 legume concentrates and their corresponding raw flours, as well as purified soyabean globulins (Tukur *et al,* 1993).

Plasma levels of triglycerides and glucose were measured enzymatically (bioMérieux, Marcy-l'Étoile, France) using an automatic system (Isamat, Lisabo, Morangis, France). Antibody titres were determined by the passive haemagglutination test (Herbert, 1973).

Statistical analyses

Digestibility results were subjected to analysis of variance and means were ranked according to the test of Scheffé, but Fiedman's test was used where variances were not homogeneous. Comparison between ileal and faecal digestibilities was carried out by the *U*-test. Skin responses were analyzed by the Kruskal–Wallis test (Dagnélie, 1970). Significance was declared at $P \leq 0.05$. AA composition of proteins were compared 2 by 2 by calculating the χ^2 distance (Guilloteau *et al,* 1983). The χ^2 distance between 2 proteins *i* and *j* was calculated as follows:

$$\chi^2 = 17 \sum_{k=1}^{k=17} (AA_{ik} - AA_{jk})^2 / ((AA_{ik} + AA_{jk})/2)$$

where AA_{ik} and AA_{jk} are the percentages of AA_k in the sum of the assayed AA in proteins *i* and *j*; *k* represents the different AA and varies between 1 and 17. As the χ^2 distance decreases, the similarity between the proteins increases.

The proportions of dietary, endogenous and bacterial protein, which could be the main constituents of digesta protein, were assessed by the method developed by Duvaux *et al* (1990). This method uses a multiple regression analysis to establish the theoretical mixture which minimizes the χ^2 distance with regard to the AA compositon of digesta. The mean composition of axenic lamb faeces (Combe, 1976) and calf meconium (Grongnet *et al,* 1981) was used as a model of undigested endogenous protein. The mean composition of pig (Mason *et al,* 1976) and sheep (Mason, 1979) faecal bacteria was used to represent the composition of gut bacteria. The common protein escaping digestion in the small intestine of calves given diets based on milk, fish or soyabean protein (Guilloteau *et al,* 1986), was used as a model of the mixture of undigested endogenous and bacterial proteins. Reference AA compositions of the 2 legume proteins were those of major globulins: glycinin (Okubo *et al,* 1969), β-conglycinin (Koshiyama, 1968) and the acidic and basic subunits of glycinin (Staswick *et al,* 1984; Hirano *et al,* 1985; Momma *et al,* 1985a,b; Negoro *et al,* 1985) for soyabean; and conglutins α and β (Duranti *et al,* 1981) for the lupin proteins.

RESULTS

Health conditions were generally satisfactory during both trials. The cannulae remained functional throughout the second experiment.

Experiment 1

Feed intake, liveweight gain and nutrient digestibility

Feed intake was very satisfactory for animals on the control and soyabean diets. In the former group, only 1 animal refused 10% of feed offered, while in the latter there were no refusals. More refusals were observed with the lupin diet where 3 animals refused 4, 15 and 35% of feed offered, respectively. Liveweight gain (LWG) of animals on the soyabean and the lupin diets were 7 and 28% lower, respectively, compared to the control diet (mean ± SE: 1 064 ± 89, 825 ± 57, and 1 143 ± 82 g/d, respectively). However, significant differences were observed only between the control and lupin diets. Consequently, DM intake/kg LWG was 16 and 31% higher with the soyabean and lupin diets, respectively, compared with the con-

trol diet. The differences were however not significant.

The soyabean and lupin diets both resulted in significantly lower digestibility values than the control diet (table IV). Faecal digestibilities of DM, organic matter (OM), nitrogen-free extract (NFE) and ash tended to be lower with the lupin diet compared to the soyabean diet, but the differences were not significant.

Triglyceride and glucose plasma levels

In control calves, plasma concentration of triglycerides tended to decrease during 4 h after the morning meal (fig 2A). It then increased during the next 2.5 h, but stayed lower than preprandial levels. With the soyabean and lupin diets, preprandial levels of triglycerides were significantly lower compared to the control values. However, they increased steeply during the first 2 h after the meal to significantly higher levels than with the control diet. They then decreased during the next 4.5 h particularly with the lupin diet, whose value at 6.5 h was significantly lower than that of the control and soyabean diets. With the soyabean diet, the levels observed remained significantly higher up to the fourth hour after the meal.

Table IV. Apparent ileal and faecal digestibilities (means and SE).

Level	*Diet*					
	Control		*Soyabean*		*Lupin*	
	Ileal	*Faecal*	*Ileal*	*Faecal*	*Ileal*	*Faecal*
Dry matter						
Mean	0.905[a]	0.961[a*]	0.,848[a]	0.890[b]	0.771[b]	0.840[b*]
SE	0.010	0.004	0.022	0.009	0.007	0.010
Organic matter						
Mean	0.917[a]	0.967[a*]	0.867[a]	0.908[b*]	0.791[b]	0.856[b*]
SE	0.009	0.003	0.020	0.008	0.006	0.010
Nitrogen						
Mean	0.918	0.945[a*]	0.899	0.861[b*]	0.884	0.878[b]
SE	0.004	0.004	0.012	0.010	0.004	0.013
Fat						
Mean	0.891	0.938[a*]	0.816	0.872[b]	0.905	0.876[b]
SE	0.018	0.022	0.068	0.078	0.018	0.027
N-free extract						
Mean	0.928[a]	0.986[a*]	0.871[a]	0.935[b*]	0.705[b]	0.830[b*]
SE	0.011	0.007	0.019	0.037	0.011	0.062
Ash						
Mean	0.750[a]	0.880[a*]	0.627	0.678[b]	0.527[b]	0.645[b*]
SE	0.032	0.013	0.052	0.020	0.030	0.023

[a,b,c] At a given site (ileal or faecal) $P \leq 0.05$ between values followed by unlike letters. * $P \leq 0.05$ between ileal and faecal values for the same diet.

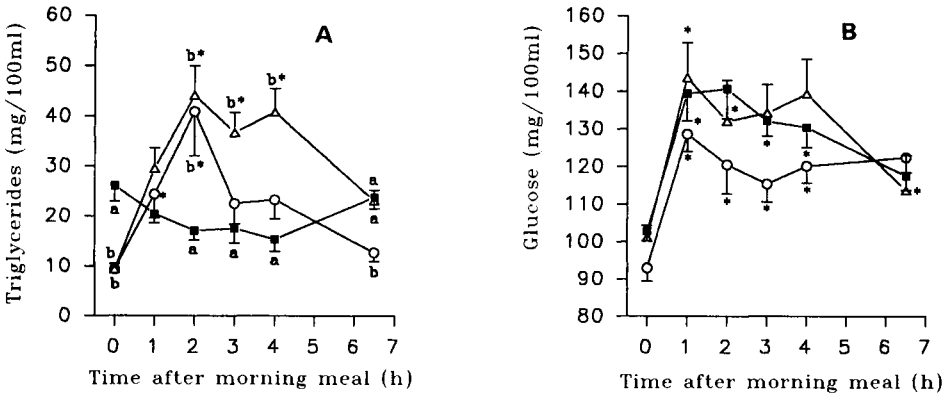

Fig 2. Effect of diet on postprandial plasma concentration of triglycerides (A) and glucose (B) for animals on control (■), soyabean (Δ), and lupin (O) diets. ^{a,b} $P \leq 0.05$ between diets, * $P \leq 0.05$ compared to preprandial values. Vertical bars are standard errors.

Plasma glucose concentration was similar among the 3 diets before the morning meal. Instantaneous (fig 2B) and cumulative (not shown) postprandial changes observed with the 3 diets over 6.5 h were similar. Glucose levels increased significantly 1 h after the morning meal, and remained higher than preprandial levels 6.5 h later for the 3 diets. The highest levels were observed 2 and 1 h after the morning meal with the control and the legume diets, respectively. During the first 4 h after feeding, glucose levels observed with the lupin diet were lower than those observed with the control and soyabean diets but the differences were not significant.

Antibody responses and skin reactions

Haemagglutination test on plasma samples revealed that none of the calves appeared to have produced dietary protein specific antibodies. The diameter of oedema recorded 1 h after histamine injection was 29, 31 and 21 mm with animals on the control, soyabean and lupin diets, respectively. This reaction had disappeared at 4 h. As expected, no skin reaction was observed with physiological saline. Dermal reactions against the test proteins observed 4 h post-injection were mostly insignificant, and virtually disappeared 8 h later (fig 3). In fact only the soyabean concentrate (1 mg) elicited a significant (though very low) response 4 h post-injection in animals on the lupin diet. A similar response was observed with milk protein (5 mg) in the same group of calves. Changes in double skin fold thickness were very low and insignificant in all treatments.

Experiment 2

Nutrient digestibility

Apparent ileal digestibilities of nutrients were lower with the soyabean diet than with the control diet (table IV), but the differences were not significant. The same trend was observed between the control diet and the lupin diet, but the differences were larger and significant ($P \leq 0.05$) except for N and fat. Furthermore, digestibilities of DM, OM and NFE were lower ($P \leq 0.05$) with the lupin diet than with the soyabean diet. With the 3 diets, digestibilities of DM, OM, NFE and

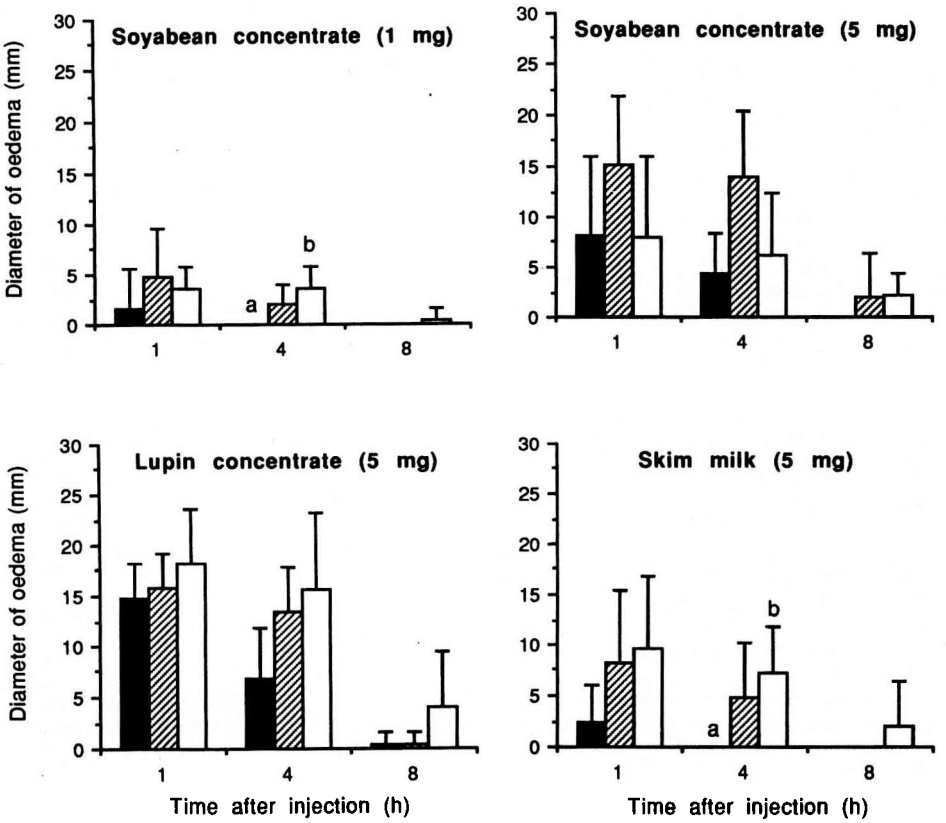

Fig 3. Skin responses of calves following the injection of the 2 protein concentrates and skim-milk protein for animals on control (■), soyabean (▨), and lupin (□) diets. [a,b] $P \leq 0.05$ between means with different letters. Vertical bars are standard errors.

minerals were higher at the faecal level than at the ileal level. N digestibility was higher at the faecal level with the control diet, but was lower with the soyabean diet ($P \leq 0.05$). Similarly, fat digestibility was higher ($P \leq 0.05$) at the faecal level with the control diet.

Apparent ileal digestibilities of assayed AA were lower with the soyabean diet than with the control diet, except for glycine, alanine, cystine and arginine (table V). The differences were however only significant for valine and tyrosine. The lupin diet also resulted in lower digestibility of AA than the control diet ($P \leq 0.05$ for proline, valine, methionine, leucine and lysine) except for glycine, cystine and arginine. The only noticeable difference between the 2 legume diets was in the case of lysine whose digestibility was lower ($P \leq 0.05$) with the lupin diet than with the soyabean diet. Digestibility of amino acid N (AAN) was also lower for the soyabean and lupin diets than for the control diet, but the differences were not significant. With the 3 diets, the apparent digestibility was higher for total AAN than for total N; differences were 0.015, 0.016

Table V. Ileal digestibilities of AA: apparent (mean ± SE) and true values.

	Apparent			*True* *	
	Control	*Soyabean*	*Lupin*	*Soyabean*	*Lupin*
ASP	0.927 ± 0.01	0.913 ± 0.01	0.913 ± 0.00	0.964	0.967
THR	0.878 ± 0.00	0.868 ± 0.02	0.865 ± 0.01	0.978	0.992
SER	0.922 ± 0.00	0.906 ± 0.02	0.892 ± 0.01	0.988	0.975
GLU	0.941 ± 0.01	0.915 ± 0.02	0.885 ± 0.01	0.986	0.946
PRO	0.956 ± 0.00[a]	0.896 ± 0.02	0.884 ± 0.00[b]	0.978	0.973
GLY	0.842 ± 0.01	0.869 ± 0.02	0.870 ± 0.01	0.957	0.963
ALA	0.893 ± 0.01	0.895 ± 0.01	0.877 ± 0.01	0.973	0.975
CYS	0.789 ± 0.01	0.854 ± 0.02	0.797 ± 0.02	0.954	0.908
VAL	0.946 ± 0.00[a]	0.906 ± 0.01[b]	0.897 ± 0.00[b]	0.976	0.974
MET**	0.963 ± 0.00[a]	0.906 ± 0.01	0.854 ± 0.02[b]	0.967	0.971
ILE	0.955 ± 0.00	0.936 ± 0.01	0.931 ± 0.01	0.985	0.982
LEU	0.957 ± 0.00[a]	0.937 ± 0.01	0.930 ± 0.00[b]	0.986	0.980
TYR	0.952 ± 0.00[a]	0.917 ± 0.01[b]	0.936 ± 0.01	0.987	0.989
PHE	0.950 ± 0.00	0.935 ± 0.01	0.925 ± 0.01	0.987	0.984
HIS	0.943 ± 0.00	0.916 ± 0.01	0.877 ± 0.01	0.978	0.951
LYS**	0.929 ± 0.00[a]	0.912 ± 0.01[a]	0.857 ± 0.00[b]	0.996	0.998
ARG	0.934 ± 0.00	0.959 ± 0.01	0.966 ± 0.00	0.996	0.992
AAN	0.933 ± 0.00	0.915 ± 0.01	0.907 ± 0.00	0.982	0.974

* Values calculated assuming that the true digestibility of milk protein was complete and that the amounts of AA per kg DM intake escaping digestion in the small intestine with the control diet corresponded to the endogenous contributions with the other diets. [a,b] $P \leq 0.05$ between values followed by unlike letters. ** Supplement excluded.

and 0.023 for the control, soyabean and lupin diets, respectively. Irrespective of the diet, the digestibilities were always higher for isoleucine, leucine, tyrosine, phenylalaline and arginine, and lower for threonine, glycine, alanine and cystine than for AAN and mean AA digestibility values with the 3 diets.

AA composition of protein sources and ileal digesta

The soyabean and lupin concentrates contained more aspartic acid, glycine, alanine, cystine and arginine, and less proline, valine, methionine, isoleucine, leucine and lysine than SMP (table III). As expected, differences between the soyabean and the lupin diets were lower ($\chi^2 = 40$) than those between the control and the 2 legume diets ($\chi^2 \geq 154$).

Irrespective of the diet, AAN represented a lower proportion of total N in ileal digesta than in the diets: 66, 66 and 65% instead of 82, 83 and 85%, with the control, soyabean and lupin diets, respectively. Compared with the diets, digesta protein contained more threonine, serine, glycine, alanine and cystine, and less methionine, isoleucine, leucine, phenylalanine (and arginine with the soyabean and lupin diets). The differences in AA profile between digesta and corresponding diets or protein sources were important as shown by large χ^2 distances ($\chi^2 \geq 129$, table VI). In addition, digesta AA composition of the 2 legume protein diets was very different from that of their major proteins and their subunits ($\chi^2 \geq 214$).

Significant differences in the proportions of aspartic acid, glycine and methionine were observed in ileal digesta between the control and the soyabean diets (fig 4); with the lupin diet, differences ($P \leq 0.05$) were observed for threonine, cystine, histidine and lysine.

Table VI. χ^2 distances between ileal digesta, dietary, endogenous, bacterial and theoretical protein mixtures *.

	Digesta		
	Control	*Soyabean*	*Lupin*
Diet			
Control	227	278	265
Soyabean	123	129	175
Lupin	201	228	205
Digesta			
Control	–	28	72
Soyabean	28	–	86
Lupin	72	86	–
Soyabean concentrate (Sc)	161	161	180
Lupin concentrate (Lc)	325	357	265
Endogenous protein (E)	132	120	276
Bacteria (B)	204	156	336
Undigested ileal mixture of E and B proteins (MEBP)	38	29	134
Glycinin	–	214	–
Subunits of glycinin			
A1	–	279	–
B2	–	303	–
β-conglycinin	–	533	–
Subunits of β-conglycinin			
α	–	574	–
β	–	549	–
Conglutin-α	–	–	347
Conglutin-β	–	–	544
81% MEBP + 19% control diet	28	–	–
82% MEBP + 18% Sc	–	31	–
93% MEBP + 7% Sc	–	29	–
85% MEBP + 15% Lc	–	–	114
89% MEBP + 11% conglutin-α	–	–	120
92% MEBP + 8% conglutin-β	–	–	122
87% control digesta + 13% Lc	–	–	65
90% control digesta + 10% conglutin-α	–	–	67
90% control digesta + 7% conglutin-β	–	–	68

* $P < 0.05$ for the fit between ileal digesta and retained theoretical protein mixtures. Endogenous protein: mean composition of axenic lamb faeces (Combe, 1976) and calf meconium (Grongnet *et al*, 1981). Bacteria: mean composition of pig (Mason *et al*, 1976) and sheep faecal bacteria (Mason, 1979). Undigested ileal mixture of E and B: common protein escaping digestion in calves given diets based on milk, fish or soyabean protein (Guilloteau *et al*, 1986). Glycinin: Okubo *et al* (1969); β-conglycinin: Koshiyama *et al* (1968); conglutins α and β: Duranti *et al* (1981); subunits of glycinin: Staswick *et al* (1984); Hirano *et al* (1985); Momma *et al* (1985a,b); Negoro *et al* (1985).

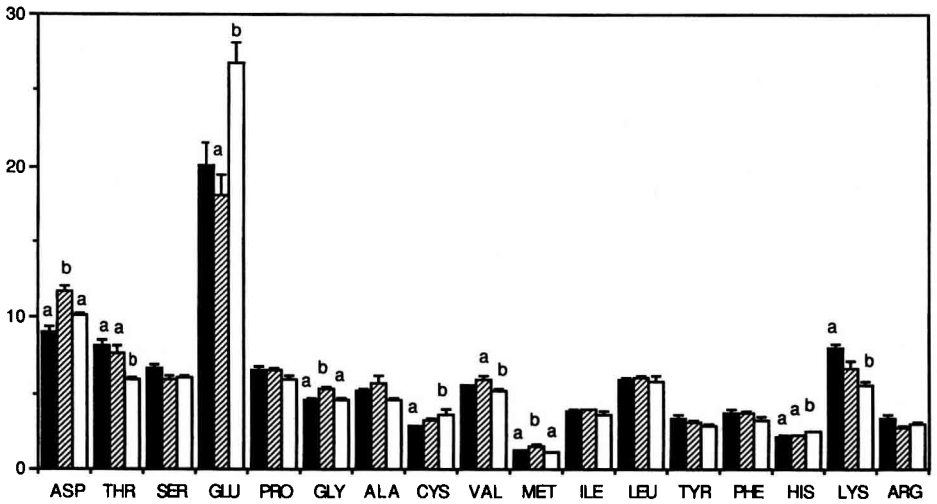

Fig 4. Amino-acid composition (% of assayed AA) of ileal digesta with the control (■), soyabean (▨) or lupin (□) diets. ^{a,b} $P \leq 0.05$ between means with different letters. Vertical bars are standard errors.

The AA composition of ileal digesta was also different from that of endogenous and bacterial protein ($\chi^2 \geq 120$). The soyabean digesta was more similar to the control digesta ($\chi^2 = 28$) than the lupin digesta ($\chi^2 = 72$). Digesta from the 2 legume protein diets differed significantly in their composition of aspartic acid, threonine, glutamine, glycine, valine, methionine and histidine. This was reflected by a relatively high χ^2 distance (86).

The amounts of AA recovered at the end of the ileum relative to DM intake are presented in table VII. The undigested amounts were always higher with the soyabean diet compared to the control diet, but the differences were not significant. The same trend was observed between the lupin and the control diet, but the differences were significant for aspartic acid, glutamic acid, cystine and histidine. No noticeable differences were observed between the 2 legume proteins. The additional undigested fractions (calculated as the total amounts of AA recovered at the end of the ileum with the 2 legume diets minus those obtained with the control diet) were rich in aspartic and glutamic acids which represented 36.3 and 54.1% of the total AA with the soyabean and lupin diets, respectively. They were very different from the dietary, whole digesta, endogenous, bacterial, soyabean and lupin proteins ($\chi^2 > 300$).

DISCUSSION

Animal performance

Overall performance was lower for animals on the 2 legume diets than on the control diet. The LWG of animals on the soyabean diet was however satisfactory, since it was similar to that obtained with a non-antigenic soyabean concentrate (Lallès *et al*, 1994). The lower LWG of animals on the lupin diet compared to those on the control diet could be explained by differences recorded in feed intake and digestibility.

Table VII. Amount (mg/kg DM intake) of apparently undigested AA (mean ± SE) and composition of the additional undigested protein (% of assayed AA).

	Diet			*Additional undigested protein* *	
	Control	*Soyabean*	*Lupin*	*(Soy)-(Control)*	*(Lup)-(Control)*
ASP	1 280 ± 51[a]	2 181 ± 451	2 059 ± 87[b]	22.09	12.55
THR	1 147 ± 24	1 370 ± 186	1 223 ± 72	5.47	1.23
SER	947 ± 29	1 086 ± 185	1 228 ± 70	3.41	4.52
GLU	2 911 ± 426[a]	3 488 ± 853	5 485 ± 457[b]	14.14	41.52
PRO	933 ± 79	1 185 ± 202	1 213 ± 28	6.19	4.52
GLY	657 ± 58	985 ± 180	922 ± 38	8.05	4.27
ALA	733 ± 43	982 ± 109	917 ± 49	6.13	2.98
CYS	398 ± 26[a]	583 ± 80	727 ± 60[b]	4.54	5.31
VAL	783 ± 52	1 055 ± 145	1 048 ± 49	6.68	4.28
MET	172 ± 12	264 ± 27	219 ± 18	2.28	0.77
ILE	535 ± 26	705 ± 115	719 ± 53	4.16	2.97
LEU	836 ± 43	1 080 ± 160	1 178 ± 81	6.00	5.52
TYR	483 ± 22	565 ± 91	585 ± 52	2.13	1.74
PHE	525 ± 22	660 ± 94	664 ± 61	3.30	2.23
HIS	302 ± 19[a]	409 ± 69	503 ± 26[b]	2.61	3.24
LYS	1 128 ± 48	1 190 ± 151	1 134 ± 48	1.48	0.16
ARG	461 ± 12	517 ± 112	597 ± 41	1.36	2.19

* Calculated as the total amounts of AA recovered at the end of the ileum with the 2 legume diets minus those obtained with the control diet. Soy: soyabean diet. Lup: lupin diet. [a,b] $P \leq 0.05$ between values followed by unlike letters.

Digestibility and AA composition

Apparent ileal digestibility was lower with the 2 milk substitute diets than with the control diet, which agrees with previous observations on soyabean products (Guilloteau *et al,* 1986; Nunes do Prado *et al,* 1989a; Caugant *et al,* 1993a). The lower ileal digestibilities of DM and OM with the soyabean and especially the lupin diets were probably due to their high content of carbohydrates which were not much degraded in the small intestine. However, assuming that ileal N digestibility for whey was 94% that of SMP (Caugant *et al,* 1993b), values calculated for the soyabean and lupin concentrates were found to be satisfactory (0.91 and 0.89, respectively). They were higher than the value obtained with an antigenic soyabean flour (0.80, Caugant *et al,* 1993a) and were similar to those observed with non-antigenic alcohol-treated soyabean concentrates (0.84, Caugant *et al,* 1993a; 0.90, Guilloteau *et al,* 1986) or a partially hydrolysed soyabean isolate (0.91, Nunes do Prado *et al,* 1989a).

The higher faecal digestibilities of DM, OM, NFE and ash compared to their ileal values were in line with earlier reports (Guilloteau *et al,* 1986; Nunes do Prado *et al,* 1989a). However, the lower faecal N digestibility observed with the soyabean diet compared to its ileal value was not expected, because a reverse change has generally been observed. This suggests that the higher content of carbohydrates in the legume concentrates used in the present experiment led to increased proliferation of

microorganisms, thus resulting in increased synthesis of bacterial N from the endogenous supply in the hind gut. A similar trend has been observed with pea flour (Nunes do Prado *et al,* 1989a; Bush *et al,* 1992). Assuming that faecal N digestibility for whey was 97% that of SMP (Grongnet *et al,* 1981), values calculated for the soyabean and lupin concentrates (0.84 and 0.86, respectively) were lower than at the end of the ileum. However, they still compared well with values reported for non-antigenic alcohol-treated (0.89, Guilloteau *et al,* 1986; 0.81 Lallès *et al,* 1994) or water-extracted (0.81, Lallès *et al,* 1994) soyabean concentrates; and were higher than those observed with antigenic or non-antigenic heated soyabean flours (0.66 and 0.76, Lallès *et al,* 1994). Thus proper denaturation and partial hydrolysis of protein appeared to improve ileal and faecal apparent N digestibility of soyabean and probably lupin products.

The lower percentage of AAN in digesta compared to the diets could be due to larger contents of hexosamines and urea which are abundant in digestive secretions and desquamated cells (Combe *et al,* 1980; Souffrant, 1991). The lower apparent digestibility of most AA with the soyabean and lupin diets than with the control diet agrees with results obtained by other workers with milk substitute diets containing non-milk protein sources such as bacteria (Guilloteau *et al,* 1980; Sedgman *et al,* 1985), fish, soyabean (Guilloteau *et al,* 1986; Nunes do Prado *et al,* 1989a, Caugant *et al,* 1993b), or pea (Bush *et al,* 1992). However, as observed for total N, AA digestibilities with the 2 legume proteins were relatively higher than values obtained with vegetable protein-based diets (Guilloteau *et al,* 1986; Nunes do Prado *et al,* 1989a). The relatively high digestibilities of cystine and arginine with the 2 legume diets compared to the control diet might be due to the higher contents of the legume proteins in these AA. The low apparent digestibility of threonine and glycine with all diets may be due to the high content of these AA in endogenous protein (Sauer *et al,* 1977; Guilloteau *et al,* 1986). The true digestibility of AA from the soyabean and lupin diets was calculated assuming that the true digestibility of milk AA was complete and that the endogenous fraction was similar with the 3 diets (table V). The values obtained were generally higher (by 0.03 for AAN) than those reported for a non-antigenic alcohol-treated soyabean concentrate (Guilloteau *et al,* 1986), thus indicating a slightly better AA utilization of the 2 legume concentrates used in this experiment.

As expected, when calves were fed the control diet, the AA composition of the digesta was very different from that of whole diet, bacteria or endogenous protein. It was however very similar to those previously obtained in our laboratory with skim-milk-based diets ($\chi^2 = 15$ when compared to the mean of 6 experiments) (Guilloteau *et al,* 1980, 1986; Bush *et al,* 1992; Caugant *et al,* 1993a,b). It was also close to the common mixture of endogenous and bacterial proteins (MEBP) escaping digestion in the small intestine (Guilloteau *et al,* 1986) of calves given diets based on milk, fish or soyabean protein ($\chi^2 = 38$) (table VI). The theoretical mixture of MEBP and control diet that showed the best fit with the control digesta contained 19% ($P \leq 0.05$) control diet ($\chi^2 = 28$), thus suggesting that a small amount of milk protein could be present in the control digesta. Similar trends were observed in digesta of calves fed skim-milk-based diets (Bush *et al,* 1992; Caugant *et al,* 1993a,b).

The AA composition of ileal digesta was little altered by the replacement of the control diet with the soyabean diet, which might explain the high true ileal digestibility of the latter, as observed with a highly digestible partially hydrolysed soyabean isolate (Nunes

do Prado *et al,* 1989a). In contrast, soyabean flour and an alcohol-treated concentrate were found to induce larger changes in digesta composition (Guilloteau *et al,* 1986; Khorasani *et al,* 1989; Caugant *et al,* 1993a). The theoretical mixture of the control digesta and the soyabean diet closest to the soyabean digesta contained only 7% ($P > 0.05$) of the soyabean concentrate ($\chi^2 = 27$). This would suggest that the lower apparent N digestibility of the soyabean diet might be more due to increased losses of undigested endogenous and bacterial proteins than to an incomplete digestion of dietary protein. However, the AA composition of the additional undigested proteins due to the soyabean diet was very different from whole dietary protein or their subunits, endogenous or bacterial proteins ($\chi^2 > 300$). In addition, no theoretical mixture of these proteins could be found to give a satisfactory fit with the additional undigested fraction. This would therefore exclude the presence of large proportions of intact proteins (of dietary, endogenous or bacterial origin) or their subunits in the undigested supplement. Therefore, small amounts of dietary protein fragments with AA profiles different from that of intact dietary protein probably escaped digestion. These fragments contained more aspartic acid and less glutamic acid than previously observed with alcohol-treated concentrates (Guilloteau *et al,* 1986; Caugant *et al,* 1993a) or a partially hydrolysed isolate (Nunes do Prado *et al,* 1989a). Only aspartic acid and cystine appeared to be of relatively low apparent availability.

Larger changes in AA composition of digesta were induced by replacing the control diet with the lupin diet. The theoretical mixture of control digesta and the lupin concentrate which gave the best fit with the lupin digesta contained 13% lupin concentrate ($P \leq 0.05$), but the χ^2 distance (65) was still too high to consider this model as satisfactory. The additional undigested protein due to the lupin diet was also very different from whole dietary proteins or their subunits, endogenous or bacterial proteins, as well as from their theoretical mixtures ($\chi^2 > 600$). As with the soyabean diet, small amounts of dietary fractions with AA profiles very different from that of intact lupin protein appeared to escape digestion in the small intestine. Glutamic acid and cystine were the only AA which presented a relatively low apparent availability. The utilization of more specific methods like the ^{15}N-technique (Souffrant, 1991) and the direct assay of dietary protein in digesta (Tukur *et al,* 1993) might be more appropriate for the quantification and identification, respectively, of residual dietary protein in digesta with the soyabean and lupin diets.

Plasma levels of triglycerides and glucose

Postprandial changes in plasma levels of triglycerides and glucose observed with the control diet are in agreement with previous observations (Toullec *et al,* 1979; Beynen and Van Gils, 1983; Nunes do Prado *et al,* 1989b). Thus despite the high contents of lipids reaching the duodenum, the circulating level of triglycerides remained unchanged or decreased with the control diet. This has been explained to be due to increased pancreatic secretion of insulin (Grizard *et al,* 1982). The drastic postprandial increase in plasma concentrations of triglycerides when the 2 legume diets were fed indicates an increased gastric emptying rate for lipids due to the lack of coagulation. The more rapid increase in postprandial levels of glucose compared to that of triglycerides observed with the 3 diets was in agreement with the more rapid gastric evacuation of lactose compared to lipids (Toullec *et al,* 1979). The more rapid decrease in plasma levels of triglycerides with the lupin diet after 2 h compared with

the soyabean diet would suggest either a slower abomasal emptying or a faster uptake of this nutrient by the tissues when the lupin diet was fed. The lower plasma concentration of glucose recorded with the lupin diet between 1 and 4.5 h could be due to the same reasons, but also to the lower lactose content in the lupin diet (32% DM) compared to the soyabean (39%). Finally, in the present experiment, the 2 legume diets did not induce gastric stasis immediately after feeding as observed by Sissons and Smith (1976) with preruminant calves fed antigenic soyabean diets. This could be due to the low antigenicity of the legume protein concentrates used in the present experiment in addition to the short experimental periods.

Antibody responses and skin reactions

Animals did not develop circulating antibodies against dietary proteins. A similar trend was observed with non-antigenic products such as hydrolysed soya protein isolates and alcohol-treated concentrates (Nunes do Prado *et al,* 1989b; Lallès *et al,* 1994). Similarly skin reactions against dietary proteins were minor and mostly insignificant, contrary to what has been observed with less refined soyabean products (Heppell *et al,* 1987; Lallès *et al,* 1993b). The only significant reaction against the soyabean concentrate observed with animals on the lupin diet was also recorded with SMP. This could be due to a number of reasons among which we can envisage: (1) a non-specific reaction as observed with SMP-fed calves skin tested with HSF (Heppell *et al,* 1987; Lallès *et al,* 1993b); (2) cross-recognition of legume proteins by antibodies; (3) a true response elicited by the earlier 2 weeks' feeding of the soyabean concentrate. What should be noted however is that skin reactions were rather limited, which is consistent with the lack of antibody detection and high digestibility values (and also the low *in vitro* immunoreactivity of the soyabean concentrate). Thus, the processing method used for the concentrates in this experiment seemed effective in inactivating antigenic factors, which agrees with their relatively high digestibilities.

CONCLUSION

The replacement of 71% milk protein by soyabean or lupin proteins resulted in a decreased apparent ileal and faecal digestibilities of nutrients, which might be partly due to increased gastric emptying rate of the legume-based diets and the insufficient elimination of their carbohydrates. Digestibilities of N and AA were however satisfactory with the 2 legume diets since they were similar to values observed with alcohol-treated soyabean concentrates. This might be due, in part, to the effectiveness of the processing method to denature dietary proteins, resulting in their increased sensitiveness to digestive secretions. Both legume concentrates had low antigenicity *in vitro* and *in vivo*, since circulating antibodies were not detected and skin reactions of animals against different test proteins were minimal. However, inclusion of lupin proteins in the diets of preruminant calves might be limited by reduced palatability which may lower animal performance.

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