

The effect of giving feeds containing soya-bean meal treated or extracted with ethanol on digestive processes in the preruminant calf

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1. Preruminant calves, fitted with abomasal and re-entrant ileal cannulas, were given, at intervals of 2–3 d by infusion into the abomasum, a series of five single experimental feeds containing heated soya-bean flour (product B) as the only protein source. The calves were sensitized in this way to a constituent in the soya beans and by the fifth feed showed a number of digestive disturbances. Further test feeds were then given in which heated soya-bean flour was sometimes replaced by soya-bean products prepared under laboratory or commercial conditions by treating 1 part fat-free raw soya-bean meal with either 1 part (product M) or 4 parts (product L) ethanol (750 ml/l) at 78–80° and evaporating the whole mixture to dryness, or by extracting 1 part meal with 4 parts aqueous ethanol under similar conditions (product K). Products M, L and K were heated with steam and finely ground. Feeds prepared from casein and from commercial ethanol-extracted concentrate (product D) were also examined.

2. Measurements were made of transit time through the small intestine, flow rate of ileal digesta, recovery of polyethylene glycol (a water-soluble marker added to the feed) and net nitrogen absorption up to the distal ileum. Compared to feeds based on casein, feeds containing products K, L or M showed some differences in digesta movement and N uptake, but much less disturbance than feeds containing product B. Products prepared by extracting soya-bean meal with ethanol appeared to be slightly superior to those prepared from meal treated with ethanol, but the differences were not significant.

3. Weanling rats were used to assess the nutritive value of soya-bean products by an N balance method. Digestibilities (mean 0.948) and biological values (mean 0.860) obtained for products B, D, K and L did not differ significantly.

4. Results confirmed that extracting soya-bean meal with hot aqueous ethanol improved its value for calf feeding and indicated that at least part of the effect was due to the destruction of a toxic constituent present in the soya bean which probably acted by inducing a gastrointestinal allergy.

Growth and digestibility trials have shown that liquid feeds containing heated soya-bean flour as the only or major source of protein are not suitable for rearing preruminant calves (Gorrill & Thomas, 1967; McGilliard *et al.* 1970; Nitsan *et al.* 1972; Kwiatkowska, 1973; Ramsey & Willard, 1975). In cannulated calves such feeds were shown to lead to inhibition of abomasal emptying, increase in rate of passage and flow of digesta through the small intestine, abnormal water and salt exchange and decreased nitrogen absorption (Smith & Sissons, 1975; Sissons & Smith, 1976). These and intact calves receiving diets containing heated soya-bean flour developed high titres of serum antibodies, and some animals showed morphological changes in the villi of the intestine (van Leeuwen *et al.* 1969; Smith & Sissons, 1975; Barratt *et al.* 1978). It seems that these disturbances in digestive function are symptoms of a gastrointestinal allergy to a constituent in the soya-bean flour.

It appears that the toxic constituent resists steam treatment which destroys most of the trypsin inhibitor and haemagglutinin activity (Birk & Gertler, 1961; Obara & Watanabe, 1971; Circle & Smith, 1972). Attempts to inactivate the toxic factor in soya-bean meal by treating it with acid or alkali have provided products of variable food value for calves (Colvin & Ramsey, 1968, 1969; Ramsey & Willard, 1975).

However, the toxic factor is apparently absent from commercial soya-bean concentrate that is prepared by extracting fat-free soya-bean meal with hot aqueous ethanol. Calves

given milk substitutes containing this product, rather than heated soya-bean flour, showed a markedly better growth and digestibility performance (Gorrill & Thomas, 1967; Gorrill & Nicholson, 1969, 1972; Nitsan *et al.* 1972). In other experiments preruminant calves fitted with intestinal cannulas and receiving ethanol-extracted soya-bean concentrate in their diet did not show marked abnormalities in digestive processes or develop serum antibodies to a preparation of soya-bean antigen (Smith & Sissons, 1975; Sissons & Smith, 1976).

The present work with cannulated calves was undertaken to determine whether hot aqueous ethanol extraction of soya-bean meal extracts or simply destroys the toxic constituent of soya-bean meal. To examine the possibility that ethanol treatment of soya-bean meal may affect the nutritive value of the protein, N-balance experiments were done with rats fed on soya-bean products treated or extracted with aqueous ethanol.

METHODS

Animals

Calves. Friesian bull calves were equipped with a simple cannula in the fundic region of the abomasum and a re-entrant cannula in the distal ileum as described by Sissons & Smith (1976). Cannulas were inserted when the animals were 2–3 weeks of age.

Calves received colostrum up to 4 d of age and then whole milk supplemented from 3 weeks with minerals and vitamins (Smith & Sissons, 1975). Except when experimental feeds were given, supplements of aureomycin (Cyanamid Agricultural Division, Gosport, Hants) were added to each feed (0.4 g/d) from 1 week after surgery.

Rats. Twelve female weaning rats of closely similar body-weight (mean 53 g) were selected from the Institute colony of Norwegian Hooded rats. They were assigned to one of three groups in a 4 × 4 Latin Square design by using a table of random numbers.

Experimental diets

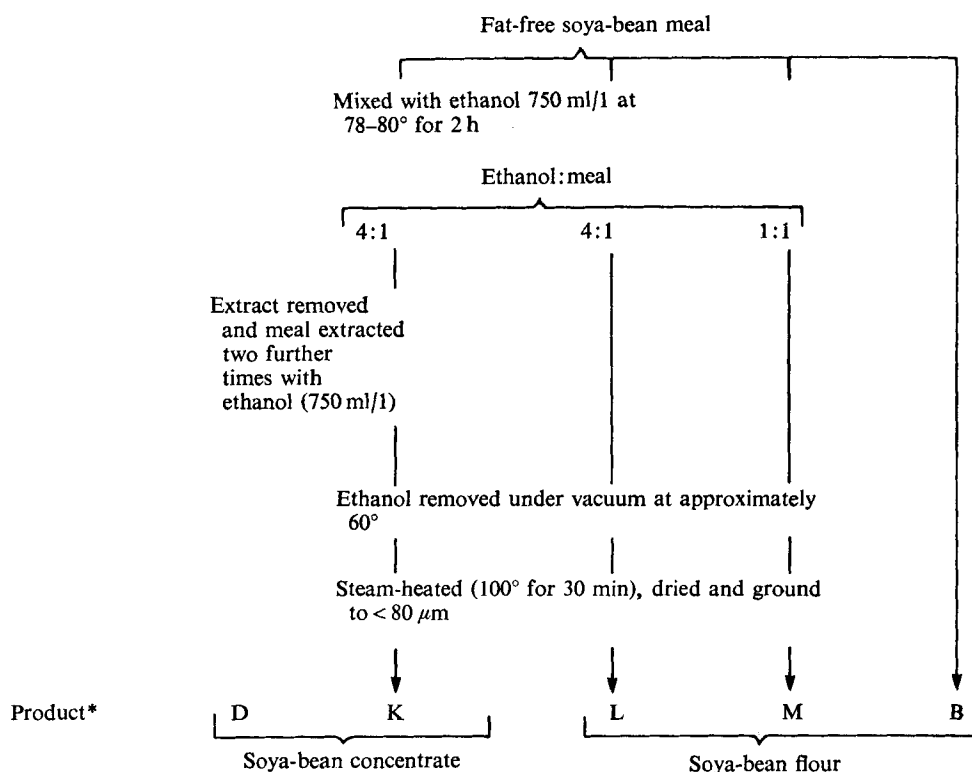
Experimental feeds were given to calves from 3 weeks after surgery when the animals were 5–6 weeks of age. The composition of these feeds has been described previously (Smith & Sissons, 1975) and included either calcium caseinate or one of the soya-bean products shown in Table 1 as the only source of protein. Amounts of soya-bean products added were (g/kg): soya-bean concentrates, products D 53 and K 55; soya-bean flours, products B 66, L 66 and M 64. All feeds contained 5 g polyethylene glycol (PEG) (molecular weight 4000) and 0.1 g phenol red as markers.

Diets B, L, D, K and E used in N-balance experiments with rats contained (g/kg): soya-bean flours, products B 153.0 and L 151.0; soya-bean concentrates, products D 116.0 and K 121.0 and fat-free egg 55.0 respectively as the only protein source. Maize starch was added in amounts 477.1, 475.1, 512.1, 507.1 and 573.1 g/kg respectively. All diets contained (g/kg): potato starch 100, margarine 100, milled sucrose 120, α -tocopheryl acetate 0.36, cyanocobalamin 0.02, choline 1.9, salt mixture 40 and vitamin supplement 9.6. The salt mixture supplied (g/kg diet): NaCl 5.6, KH_2PO_4 15.6, MgSO_4 2.3, CaCO_3 15.3, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 1.1, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.16, KI 0.032, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.022, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.019, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.001. The vitamin supplement provided (mg/kg diet): *myo*-inositol 1000, calcium pantothenate 20.0, nicotinic acid 15.0, riboflavin 3.0, pyridoxine 2.5, thiamin 2.0, *p*-aminobenzoic acid 2.0, menaphthone 2.0, pteroylmonoglutamic acid 2.0, biotin 0.2, retinol 30.0, cholecalciferol 10.0.

Digesta collections

Collections of digesta from the distal ileum of calves were made as described by Sissons & Smith (1976, 1978). In Expt 1, digesta collections were made from three calves after giving experimental feeds prepared from casein. A series of five experimental feeds containing

Table 1. Methods of processing different soya-bean products given to calves



Crude protein
(nitrogen × 6.25)
content (mg/g)

688 660–662 523–534 544 526–533

NSI

4.9 4.2–6.8 5.1–5.3 5.2 18.9–21.6

Trypsin
inhibitor
(units†/g)

3.5 4.5–5.2 3.3–6.5 4.3 6.9–8.3

NSI, nitrogen solubility index (American Oil Chemists' Society, 1965).

* Products were either prepared in the laboratory or supplied by Aarhus Oliefabrik, Denmark (products B, K, L and M). Product D was a commercial soya-bean concentrate (Danpro A) prepared by extracting fat-free soya-bean meal with hot aqueous ethanol.

† For definition of units, see Kakade *et al.* (1974).

heated soya-bean flour (product B) were then given at 2–3 d intervals and collections made after each feed. By the fifth feed disturbances in digesta flow had become apparent to a greater or lesser extent with the different calves and they were regarded as being sensitized to the toxic factor in the flour (see Smith & Sissons, 1975; Sissons & Smith, 1976). Comparisons were subsequently made between feeds containing casein and feeds containing soya-bean products B, K or L which were prepared in the laboratory as outlined in Table 1. In earlier work (Smith & Sissons, 1975; Sissons & Smith, 1976) calves receiving a series of

feeds containing heated soya-bean flour showed some variation in the extent to which they developed digestive disorders. Because of this variation it was considered necessary to establish that the calves were sensitive to heated soya-bean flour before examining the effects of feeds containing soya-bean meal which had been extracted or treated with ethanol. Also, by using this method, several different soya-bean products could be tested on the same animal; calves sensitive to heated soya-bean flour would not be expected to show tolerance to other soya-bean products containing the toxic factor (see Smith & Sissons, 1975; Sissons & Smith, 1976).

In Expt 2, five calves, sensitized with heated soya-bean flour feeds in the same way as in Expt 1, were given a casein feed and then a series of feeds at 2–3 d intervals containing soya-bean products B, D, K, L or M (prepared by Aarhus Oliefabrik, Denmark as outlined in Table 1) according to a Latin Square design (calves \times diets). The group of soya-bean product-based feeds was then repeated according to a second Latin Square. One calf was removed from this part of the experiment because of leakage of digesta from the ileal re-entrant cannula. Finally the remaining four animals were given a casein feed. Collections of digesta were made after giving each experimental feed.

N-balance experiment with rats

Biological value (BV) of soya-bean protein was estimated by the method of Mitchell (1923–4). Rats were placed singly in glass metabolism jars as described by Rolls *et al.* (1976). In these jars the urine was absorbed by filter paper (Whatman no. 3), sprinkled lightly with 0.1 M-sulphuric acid to prevent losses of ammonia, while faeces collected on the surface of the paper. Initially all twelve rats received the basal low N diet containing egg protein. After a period lasting 8 d (a 4 d adaptation period followed by a 4 d collection period), the rats received each of the four soya-bean diets B, D, K and L according to a Latin Square design (periods \times diets) during four 8 d periods. The amount of food given daily to each rat was 0.1 g/g body-weight. This food was made into a slurry with water to prevent wastage.

At the start of each collection period the rats were moved to a cage cleaned with acid and provided with fresh filter paper. Faeces and urine were collected as described by Rolls *et al.* (1976). Total collections of faeces over 4 d periods were taken up in water, homogenized and diluted to 50 ml or 200 ml with 0.1 M-H₂SO₄ for collections from rats that had received an egg or soya-bean diet respectively. Urine collected over 4 d together with acid washings from cages and filter papers were diluted to 500 ml.

Analytical methods

Total N. Samples of homogenized digesta (1 g) or faeces (2 g), liquid diet (1 g), urine (3 ml) or dietary protein source (0.1 g) were digested as described by Smith & Sissons (1975) and the ammonia-N produced was estimated colorimetrically using an automated technique (Technicon Instruments Co. Ltd, 1967).

PEG. The amount of PEG in digesta samples was determined by the method described by Smith (1958, 1962), except that 20 min was allowed for the development of turbidity. Phenol red, when it was present, was first removed from the samples according to Smith (1964).

Solubility of nitrogenous components of soya-bean protein sources. This was determined as the 'N solubility index' (NSI) as described by the American Oil Chemists' Society (1965).

Trypsin inhibitor in soya-bean protein sources. The trypsin inhibitor content was determined, and results expressed, as trypsin inhibitor units by the method of Kakade *et al.* (1974).

Table 2. *Effects of giving, by abomasal infusion, experimental feeds containing different soya-bean products* prepared in the laboratory on digestive processes in the sensitized calf †*

(Values are means with their standard errors for the three calves; the values for each calf were themselves means of three observations)

Period of collection ‡ (h) . . .	Small intestine transit time (h)		Ileal flow (g/h)		PEG recovery (proportion of intake)		Net nitrogen absorption (proportion of intake)					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE				
	3		21		3		21					
Protein source in feed	Mean	SE	Mean	SE	Mean	SE	Mean	SE				
Casein	3.9	0.5	113	23	50	7	0.62	0.05	0.94	0.03	0.84	0.01
Soya-bean product:												
B	1.4	0.2	535	152	192	51	0.22	0.07	0.82	0.03	0.16	0.19
K	2.5	0.1	281	36	66	7	0.51	0.08	0.99	0.01	0.75	0.05
L	1.9	0.2	237	37	87	22	0.49	0.04	0.96	0.01	0.58	0.15

PEG, polyethylene glycol (molecular weight 4000).

* For details, see p. 478 and Table 1.

† Experimental feeds containing soya-bean product B had been given on five previous occasions, see p. 478.

‡ Period of digesta collection during first 3 or 21 h after food residues reach the distal ileum.

Table 3. *Effect of giving, by abomasal infusion, experimental feeds containing different soya-bean products* prepared under commercial conditions on digestive processes in the sensitized calf†*

(Mean values for four calves; the values for each calf were themselves means of two observations)

Period of collection‡ (h) . . .	Small intestine transit time (h)	Ileal flow (g/h)		PEG recovery (proportion of intake)		Net nitrogen absorption (proportion of intake)
		3	21	3	21	
Soya-bean product:						
B	1.66	502	152	0.48	0.95	0.44
D	2.50	268	87	0.61	0.94	0.68
K	2.59	204	76	0.53	0.89	0.73
L	2.11	283	105	0.51	0.99	0.66
M	2.05	201	114	0.45	0.97	0.61
SE of difference§ (df)	0.22(20)	117(20)	22(18)	0.08(18)	0.06(18)	0.08(18)

PEG, Polyethylene glycol (molecular weight 4000).

* For details, see p. 478 and Table 1.

† Experimental feeds containing soya-bean product B had been given on five previous occasions, see p. 478.

‡ Period of digesta collection during first 3 or 21 h after food residues reach the distal ileum.

§ Based on the error mean square in the analysis of variance.

Statistical analysis. In Expt 2 with calves and the N-balance experiment with rats treatment differences were examined by analysis of variance. In Expt 1 with calves, because of the variation in 3 h and 21 h ileal flow and nitrogen absorption for heated soya-bean flour feeds relative to feeds containing casein, product K or product L, analysis of variance was not appropriate and the treatment differences were assessed by a significance test of paired comparisons.

RESULTS

Digesta flow and N utilization after calves were given feeds containing soya-bean products

Earlier studies (Sissons & Smith, 1976) showed that when calves were given a series of feeds containing heated soya-bean flour disturbances in the movement of digesta through, and absorption of N from, the small intestine became apparent. After four or five such feeds the severity of disorders usually stabilized and the calf was regarded as being sensitive to soya-bean flour. Both results in Expt 1 and 2 showed that when calves were given successive feeds prepared from soya-bean flour mean rates of digesta flow increased markedly between the first and fifth feed confirming earlier observations (Smith & Sissons, 1975).

Table 2 shows measurements of transit time, mean rates of digesta flow, recoveries of PEG and net disappearance of N between mouth and ileum (see Sissons & Smith, 1976) for Expt 1 in which calves were sensitized to heated soya-bean flour and then given feeds based on different soya-bean products and casein. Measurements of small intestine transit time, mean 3 and 21 h ileal flow, 3 h PEG recovery and net N absorption obtained for heated soya-bean flour (product B) were significantly different ($P < 0.05$) from those for casein- or ethanol-extracted soya-bean meal (product K). Results obtained for casein and heated soya-bean flour were closely similar to previously reported values for sensitized calves given these feeds (Sissons & Smith, 1976). Values for ethanol-treated soya-bean meal (product L) were intermediate between those obtained for heated soya-bean flour (product B) and ethanol-extracted soya-bean meal (product K), but the differences were not significant.

In Expt 2 feeds prepared from soya-bean meal commercially extracted or treated with

Table 4. *Biological values (BV) and digestibilities of proteins of different soya-bean products prepared under commercial conditions*

(Mean values for twelve rats)

Protein source*	BV	Digestibility
Soya-bean product		
B	0.860	0.946
D	0.857	0.960
K	0.849	0.950
L	0.873	0.937
SE of difference† (df)	0.027(24)	0.010(24)

* For details of diets, see p. 478.

† Based on the error mean square in the analysis of variance.

ethanol (products K, L and M) were compared with heated soya-bean flour (product B) and soya-bean concentrate (product D). Compared to values for heated soya-bean flour (Table 3) corresponding measurements for products D, K and L were significantly different ($P < 0.05$). Calves receiving product K also had significantly lower values for 3 h ileal flow than animals given product B-based feeds. Results obtained for ethanol-extracted soya-bean meal (product K) showed slightly less disturbance than those for ethanol-treated meal (product L), but the differences were not significant. When the meal treated with an equal weight of ethanol (product M) was used results did not differ appreciably from values obtained when animals received feeds containing product L. Compared to product M feeds those containing heated soya-bean flour led to lower small intestine transit time, higher 21 h ileal flow and lower 3 h PEG recovery, but these differences were not significant; and higher 3 h ileal flow and lower net N absorption which were significantly different ($P < 0.05$).

Casein feeds were given to calves before and after the Expt 2 series of feeds containing different soya-bean products. Values for small intestine transit time (h), 3 and 21 h ileal flow (g/h), 3 h and 21 h PEG recovery (proportion of intake) were (mean \pm SE): 3.0 ± 0.4 , 92 ± 25 , 60 ± 4 , 55 ± 10 , 92 ± 2 and 83 ± 2 respectively. Results similar to these were obtained in previous studies (Sissons & Smith, 1976).

It appeared from the results of Expts 1 and 2 that hot aqueous ethanol treatment of soya-bean products for calf feeding had a beneficial effect by inactivating toxic factor(s) that were responsible for disturbances of digestive processes. To check whether or not this ethanol treatment affected the BV of the protein, an N-balance experiment was done with rats receiving soya-bean products B, D, K and L in their diets. During the period of the experiment the rats gained weight at similar rates of approximately 1.0 g/d. Except for small refusals on the first day of the adaptation period, all food offered to the rats was eaten. Results of this experiment are given in Table 4. There were no marked differences between results for the digestibilities or BV of soya-bean products B, D, K and L. These measurements of BV are slightly higher than values of between 0.78 and 0.83 for heated soya-bean flour reported by other workers (Henry & Toothill, 1962; Kapoor & Gupta, 1975) and marginally lower than values of 0.88–0.90 obtained for casein in another study made in this laboratory (Hewitt & Ford, personal communication).

DISCUSSION

Previous work (Smith & Sissons, 1975; Sissons & Smith, 1976) has shown that feeding different sources of protein to preruminant calves may lead to variations in the movement of digesta through, and absorption of nutrients from, the alimentary tract. Results of the

present experiments in which calves given a series of feeds containing heated soya-bean flour showed disturbances in digestive functions confirmed earlier observations, and supported the idea that such variations are symptoms of a gastrointestinal allergy to a constituent of the soya-bean product.

Soya-bean meal extracted with hot aqueous ethanol (product K) was similar to certain commercial ethanol-extracted concentrates (Sissons & Smith, 1976) in not causing digestive disorders in calves sensitive to heated, but otherwise untreated, soya-bean flour (Tables 2 and 3). The beneficial effect of ethanol extraction of soya-bean meal used in calf diets in preventing digestive disorders and in improving the growth of calves (Gorrill & Thomas, 1967; Nitsan *et al.* 1971, 1972) may have been due in part to the removal of ethanol-soluble oligosaccharides. Sucrose, a major disaccharide in soya beans, is not digested by the calf because of the absence of sucrase (*EC* 3. 2. 1. 26) from the intestine (Siddons, 1968). Although in previous experiments sucrose added to feeds containing casein or ethanol-extracted soya-bean concentrate (product D) did not lead to any appreciable change in the flow of ileal digesta (Sissons & Smith, 1976), the possibility of a small effect due to the oligosaccharides cannot be excluded.

It can be concluded from our present and earlier results (Smith & Sissons, 1975; Sissons & Smith, 1976) that extraction of fat-free soya-bean meal with ethanol (750 ml/l) at 80° for 2 h leads to a product not causing digestive disorders when given to calves. Barratt *et al.* (1978) reported that calves given milk substitutes containing an unspecified commercial soya-bean concentrate prepared by ethanol extraction of soya meal showed high titres of antibodies to the soya-bean concentrate in their serums. This finding does not agree with our earlier observations in calves receiving a commercial soya-bean concentrate (product D) in their diet (Smith & Sissons, 1975). It is probable that differences in treatment conditions, such as ethanol concentration and period and temperature of extraction, of soya-bean products used in different laboratories may be responsible for such variations.

The absence of severe disturbances in digesta movement and N absorption in calves given feeds containing ethanol-treated soya-bean products (Tables 2 and 3) indicates that the solvent inactivates, rather than extracts, the toxic constituent. Recent immunological studies in this laboratory have identified the presence of two antigens, glycinin and β -conglycinin in fat-extracted soya-bean meal. These constituents were found to be stable to steam treatment, but lost their antigenicity when soya-bean meal was treated with hot aqueous ethanol (Kilshaw & Sissons, 1979 *a,b*). In the present work reducing the volume of the solvent from 4 parts to 1 part aqueous ethanol to 1 part soya-bean meal (product M) did not appear to impair the effectiveness of the ethanol treatment markedly (Table 3).

It is well established that soya-bean meal will not support the normal growth of rats unless it is properly heated to destroy trypsin inhibitor and haemagglutinins (Liener, 1973). Further improvements in the performance of rats has been reported when heated soya-bean meal in their diet is supplemented with methionine (Henry, 1965; Kapoor & Gupta, 1975). Since the diets used in the present N-balance experiment were not supplemented with sulphur amino acids the results for BV and digestibility determinations were somewhat higher than expected. However, there was little or no difference between the nutritive values of the ethanol-treated or -extracted soya-bean products or heated soya-bean meal. These results indicate that ethanol treatment does not affect amino acid availability in the soya-bean protein.

It is suggested that hot aqueous treatment of soya-bean meal is a simpler process than solvent extraction for the commercial preparation of soya-bean products for calf feeding and may be nearly as satisfactory.

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