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Inhibitory Action of Spray Dried Blood Plasma and Whole Egg Powder on Lectins in Extracts of Several Legume Seeds: a Qualitative Approach

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Abstract: Samples of seeds from eight legume species and *Triticum vulgaris* grains were extracted with buffer and lectin activity in the extracts was determined in haemagglutination experiments using normal or Pronase-treated rabbit erythrocytes. The effect of the addition of spray dried porcine and bovine plasma powder, whole egg powder, galactosides, whey powder and specific inhibitors (eg mannose, galactose, N-acetyl galactosamine, fetuin) on haemagglutination activity (HA) was determined. Plasma powders were potent inhibitors of HA in extracts of *Pisum sativum*, *Vicia faba*, *Vicia sativa*, *Lens culinaris* and *Phaseolus vulgaris*. HA in extracts of *Lupinus* sp and *Phaseolus vulgaris* was efficiently decreased by whole egg powder, while the lectin of *Glycine max* could only be inhibited by addition of galactosides, whole and defatted milk powder and whey powder. Inhibitors (plasma and whole egg powder and fetuin) were subjected to SDS-PAGE and Western blotting and blots were incubated with biotinylated lectins, except for *Lupinus* lectin. Results of the HA experiments were confirmed: lectins which were not influenced by inhibitory compounds in HA experiments also showed no binding with proteins of the blotted inhibitor. There were strong indications that lectins were not bound to the albumin fraction of the plasma powders. Results are discussed in view of future *in vivo* experiments. © 1998 SCI.

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INTRODUCTION

Because of their high protein content, legume seeds are considered as potentially important protein sources in rations for monogastric animals, such as piglets and fattening pigs. However, besides the fact that S-amino acids are present in suboptimal proportions, the seeds contain several antinutritional factors (ANF), which can considerably hamper digestion and utilisation of the protein by the animal (Huisman and Jansman 1991; Gatel 1994). It is now recognised that lectins are to be considered as the most important ANF in most legume seeds (Begbie and Pusztai 1989). For example, it is thought that the negative effect of feeding raw soybeans is not only due to the presence of protease inhibitors, but also the result of lectins present in the seeds (Schulze *et al* 1995). These glycoproteins recognise and bind with glycosylated receptors of epithelial cells located on the villi of the small intestinal mucosa, thus interfering in a negative manner with digestion, absorption and utilisation of nutrients (Pusztai *et al* 1991; Grant and Van Driessche 1993; D'Mello 1995). In general, unfavourable effects of ANF can be decreased by appropriate (expensive) heat treatment of the seeds,

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but in the case of lectins, part of them can remain fully reactive after thermal treatment (van der Poel 1990; Pusztai *et al* 1991). On the other hand, a thermal denaturation of lectins and protease inhibitors can finally result in lower utilisation of the protein fraction in the seeds (van Barneveld *et al* 1994). Therefore, other inactivation procedures should be investigated. Such an alternative method would be the feeding of inhibiting substances, occupying the sugar-binding sites of the dietary lectins, thus preventing them of binding to glycosyl residues of receptors on the mucosa of small intestine and thus acting as receptor analogues. This approach is analogous to the inhibition of bacterial fimbrial lectins (adhesins), resulting in a decrease or elimination of adhesion of pathogenous *E coli* to the intestinal mucosa. In this respect, glycoproteins in spray dried blood plasma powders were very effective inhibitors (Van Driessche *et al* 1995). Therefore, the possibility of neutralising the effect of lectins in the diet by addition of spray dried plasma powder and whole egg powder, as suggested by Seynaeve *et al* (1995) was investigated. In this paper, results of a series of *in vitro* experiments are presented, investigating the effect of plasma and whole egg powder on the lectin activity in seed extracts of eight legume species and wheat.

MATERIALS AND METHODS

Legume grains and lectin inhibitors

The following legume seeds were involved in these experiments: *Pisum sativum*, var Wonder van Kelvedon, var Accent, var Cantor, var Baccara (Bucomat NV, Eke, Belgium), var Carrera (Aveve, Landen, Belgium); *Vicia faba*, var Caspar, var Toret (Aveve); *Phaseolus vulgaris*, var Saxa, var Processor (Bucomat NV); *Vicia sativa*, var Carole (Bucomat NV), var Valor (Aveve); *Lens culinaris*, var unknown (Versele-Laga, Astene, Belgium), var Anicia (Agri-Obtentions, Guyancourt, France); *Lupinus angustifolius*, var Kubeza (Aveve); *Lupinus albus*, var Lutop, var Lublanc (Agri-Obtentions); *Glycine max*, var unknown (VAMO mills, Gent, Belgium), var Alaric (Agri-Obtentions). Because it was decided to use wheat as an important ingredient in legume seed rations for future *in vivo* trials, this cereal grain was also included in the screening experiments described here (*Triticum vulgaris*, var unknown).

Products tested for their eventual lectin inhibiting activity were: spray dried bovine plasma powder (75BSC; VEOS NV, Zwevezele, Belgium), spray dried porcine plasma (75PSC; VEOS NV), whole egg powder (44EP; VEOS NV) and whey powder (RADAR; Astene, Belgium). For SDS-PAGE experiments, three different badges of the porcine plasma were used (plasma 1, 2 and 3), while for bovine plasma two batches were involved (plasma 1 and 2). For haemagglutination experiments, bovine plasma 1 and porcine plasma 1 were used (Table 1).

Determination of haemagglutination activity (HA) in seed extracts

Seeds were ground in a Brabender mill (1 mm sieve; Duisburg, Germany) and aliquots (0·3–2·5 g) were extracted in 10–5 ml of saline (0·9% NaCl w/v, 20 mM $CaCl_2$, 20 mM $MnCl_2$, 0·2% NaN_3; pH 6·4) overnight in a cold room (5°C) while shaken continuously. The optimal amount of material used for extraction varied from species to species and was determined in preliminar experiments. *Triticum vulgaris* seeds were extracted in 10 ml of 0·1 M HCl. After extraction, samples were centrifuged at 10 000 × *g* (15 min; 2°C) and an aliquot (25 µl) of the supernatant was used for serial dilution in microtiter plates. Saline (0·9% NaCl w/v; pH 7·3) or phosphate buffered saline (PBS, pH 7·4; Aubrecht and Toth 1995) was used as dilution liquid. Per well 25 µl of a suspension (4% v/v in saline or PBS) of washed erythrocytes were added. Preliminar experiments using red blood cells from different animal species indicated that rabbit erythrocytes were most appropriate for the determination of HA. In order to permit detection of HA, for some legume seeds (*Vicia faba*, var Caspar; *Lupinus* sp and *T vulgaris*, red blood cells had to be pre-incubated with a protease (Pronase, Boehringer, Mannheim, Germany) before use in the agglutination test (Kim and Madhusudhan 1988). Therefore, 10 ml of washed erythrocytes (4% in PBS) were incubated for 1 h at 37°C with 0·2 mg of Pronase. Erythrocytes were centrifuged and washed with PBS and then added in the microtitre plates. Haemagglutination was checked microscopically because it was soon noticed that with extracts of seeds with a very high protein content, visual estimation of agglutination could lead to serious errors in titre determination. HA was calculated as outlined by Valdebouze *et al* (1980) and expressed as haemagglutination units mg^{-1} of seeds. The effect of spray dried porcine or bovine plasma powder, whole egg powder, milk powder (lyophilised milk), whey powder, or specific inhibitors (mannose, galactose, *N*-acetyl galactosamine, fetuin) on HA was investigated as follows: inhibitors were dissolved in saline or PBS at different concentrations (0·1–2·5 mg ml^{-1}), except for the sugars where 20 mM was used, and these solutions were then used for serial dilution of the extracts in the microtitre plates. Eventual inhibition of HA could be demonstrated by comparison with HA of the extract without any inhibitor. In the first series of experiments, serial dilution was done in triplicate or quadruplicate, but it became soon clear that duplicate dilution already gave very reliable results. In the tables, both values are given, unless they were identical. Statistical treatment on data was not done

TABLE 1
Influence of plasma powder, whole egg powder and specific inhibitors on haemagglutination activity (units mg^{-1} of seeds) in extracts of legume seeds[a,b]

Inhibitor	*Pisum sativum*					*Phas vulgaris*		*Lens culinaris*		*Glycine max*	
	(*1*)[c]	(*2*)	(*3*)	(*4*)	(*5*)	(*6*)	(*7*)	(*8*)	(*9*)	(*10*)	(*11*)
None	51–103	51–103	51–103	51–103	51–205	1024	1024	26–51	26–51	256–682	85–171
Porcine plasma											
1·0 mg ml^{-1}	6	6	6	6	6	64–128	16–128	3	3–6	341	85–171
0·5 mg ml^{-1}	—[d]	—	—	—	—	256	128–256	3	6	341–682	—
0·1 mg ml^{-1}	—	—	—	—	—	—	—	13	—	—	—
Bovine plasma											
2·5 mg ml^{-1}	13	6	13	13	13	—	—	—	—	—	—
1·0 mg ml^{-1}	6–51	26–51	26–51	26–103	26	128	128	3–6	6–13	682	171
0·5 mg ml^{-1}	—	—	—	—	—	128–256	256	13	13	341–682	—
Whole egg											
2·0 mg ml^{-1}	51	51	—	—	—	128	32	26–51	26–51	256	171–341
Mannose (20 mM)	26	26	26	26	26	256–512	256	13–26	26	341–682	85–171
Fetuin (1·0 mg ml^{-1})	51	51–103	51–103	51–103	51–103	8–16	16	13	26–51	341–682	171–341
Galactose (20 mM)	—	—	—	—	—	—	—	—	—	8–16	5
galNAc (20 mM)[e]	—	—	—	—	—	—	—	—	—	—	—

Inhibitor	*Vicia sativa*		*Vicia faba*		*Lupinus albus*		*L ang*	*Trit vulgaris*	
	(*12*)	(*13*)	(*14*)	(*15*)*	(*16*)*	(*17*)*	(*18*)*	(*19*)*	(*20*)*
None	13	13	26	41	41	41–82	5–21	20–82	3–5
Porcine plasma									
1·0 mg ml^{-1}	1	2–3	2–3	1	20–41	41–82	0·6–1·3	20	5
0·5 mg ml^{-1}	3	3	3	3	—	—	0·6	20	—
0·1 mg ml^{-1}	6	6	6–13	—	—	—	—	—	—
Bovine plasma									
2·5 mg ml^{-1}	—	—	—	—	—	—	—	—	—
1·0 mg ml^{-1}	6	6	6–13	10	20–41	41–82	0·6–1·3	20	5
0·5 mg ml^{-1}	13	6	13	20	—	—	0·6–1·3	20–41	—
Whole egg									
2·0 mg ml^{-1}	13–26	13–26	26	41–82	0·2	0·2–0·3	3	41–82	1·3
Mannose (20 mM)	6	6	6–13	5	41	41–82	10	20	3–5
Fetuin (1·0 mg ml^{-1})	6	13	13	—	—	—	—	—	—
Galactose (20 mM)	—	—	—	41	20	5–10	1·3–2·6	—	3
galNAc (20 mM)[e]	—	—	—	—	41–82	20–41	—	—	—

[a] Haemagglutination activity was determined with untreated rabbit erythrocytes (* Pronase treated rabbit erythrocytes) and calculated as outlined by Valdebouze *et al* (1980).
[b] Dilution series were done in duplo, and if not identical, both values are given. Some experiments were repeated once or twice and in this case extreme values are given, which explains the sometimes large spread of the data (different extracts).
[c] (1) *P sativum* var Wonder van Kelvedon; (2) var Carrera; (3) var Accent; (4) var Cantor; (5) var Baccara; (6) *Phas vulgaris* var Saxa; (7) var Processor; (8) *L culinaris* var unknown; (9) var Anicia; (10) *Glycine max* var unknown; (11) var Alaric; (12) *V sativa* var Carole; (13) var Valor; (14) *V faba* var Toret; (15) var Caspar; (16) *L albus* var Lutop; (17) var Lublanc; (18) *L angustifolius* var Kubeza; (19) *T vulgaris* var unknown; (20) var unknown.
[d] Not determined.
[e] N-acetyl galactosamine.

because this study should be considered only as a qualitative approach.

SDS-PAGE and Western blotting experiments

SDS-PAGE of potential inhibitors, eg plasma, egg powder and fetuin, was performed basically following Laemmli (1970), using the Mini-Protean II Vertical Electrophoresis System (Bio-Rad, Eke, Belgium). Samples (0·3% w/v in H_2O) were prepared by heating at 100°C for 5 min with an equal volume of sample solution (26% v/v Tris buffer, 0·5 M, pH 6·8; 42% v/v of SDS 10% w/v in water; 11% v/v 2-mercaptoethanol; 21% v/v glycerol), slightly coloured blue with a small

amount of bromophenol blue (Na-salt). Proteins were separated in 12% acrylamide gels and bands visualised by Coomassie blue staining. The first lane was loaded with standard proteins: phosphorylase b, 92 kDa; catalase, 60 kDa; fumarase, 50 kDa; aldolase, 40 kDa; carbonic anhydrase, 30 kDa, trypsin inhibitor, 20 kDa, lysozyme, 14·3 kDa. For gels intented for Western blotting, biotinylated standard proteins were used: phosphorylase b, 92 kDa; catalase, 60 kDa; alcohol dehydrogenase, 40 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20 kDa; lysozyme, 14·3 kDa (Sigma, St Louis, MO, USA). Separated proteins (not stained) were transferred to nitrocellulose paper (0·45 μ pore diameter, 9 × 6 cm, Schleicher and Schuell, Dassel, Germany), in a Mini Trans Blott Cell (Bio-Rad) under standard conditions overnight at 30 V, *c* 140 mA. Blots were washed with TBS (Tris 0·05 M, 0·9% NaCl, pH 7·5) and then reacted with blocking reagent (Boehringer, Mannheim, Germany) during 1 h. Blots were again washed (5 min), twice with TBS and once with incubation buffer (TBS, pH 7·5 containing 1 mM of respectively $MgCl_2$, $MnCl_2$ and $CaCl_2$). Blots were then incubated with 100–500 μg of biotinylated lectins (Sigma) in 10 ml of incubation buffer during 1 h, followed by three consecutive washings (5 min) with approx 20 ml of TBS. Blots were incubated (1 h) with 20 ml of TBS containing 2 μl of Extravidin–alkaline phosphatase (E 2636, Sigma). Then they were five times abundantly washed (5 min) with TBS. All incubations and washings were done at room temperature. Bound lectins were visualised by addition of 15 ml of colouring reagent (0·1 M Tris, 0·05 M $MgCl_2$, 0·1 M NaCl; pH 9·5) containing 75 μl of 4-nitroblue tetrazolium chloride (NBT, Boehringer; 100 mg 1·3 ml^{-1} of dimethylformamide 70% v/v) and 56 μl of 5-bromo-4-chloro-3-indolyl-phosphate (X-phosphate, Boehringer; 50 mg ml^{-1} water). When bands were visible, blots were abundantly washed with distilled water and dried between filter paper. Blots were also incubated with lectins in the presence of saturating amounts (eg mannose: 100 mM; fetuin: 50 mg) of their specific inhibitor to verify whether an eventual binding was due to a protein–sugar interaction. As biotinylated lectin of *V faba* was not commercially available, biotinylation was done as follows: 1 mg of lectin (Sigma) was incubated overnight (5°C) with 1 ml of water containing 36 mg of mannose and 25 μl of *N*-hydroxy-succinimide ester of biotin (NHS, Sigma; 8 mg ml^{-1} of DMF) and dialysed against PBS.

RESULTS AND DISCUSSION

Haemagglutination experiments

The influence of the addition of porcine and bovine plasma on HA, which is a measure of lectin activity in extracts of legume seeds, is presented in Table 1. Mannose, galactose, *N*-acetyl galactosamine and fetuin, known as specific inhibitors of the different lectins were also tested and were considered as reference inhibiting substances. Unlike the largest part of the seeds screened in the first series of experiments using untreated erythrocytes, plasma powders had no effect on HA in extracts of *Glycine max* (Table 1). Increasing the amount of plasma powder to 2–5 mg ml^{-1} dilution buffer was still without any effect (results not shown). The only inhibitory compound was galactose, as earlier reported (Grant 1991). HA in extracts of the other seeds was considerably decreased by addition of plasma powders and it seemed that porcine plasma was a more potential inhibitor than bovine plasma. The latter had no effect on *P sativum*, when added to the dilution buffer at a concentration of 1 mg ml^{-1}, except for var Baccara, but an increase to 2·5 mg ml^{-1}, resulted in clear decreases of HA. Even in extracts of *Phas vulgaris*, having a much higher HA than the other seeds, plasma powders acted as potent inhibitors. Assuming that lectins were completely extracted, the equivalent amount of seed meal present in a well on the microtiter plate could be calculated, taking into account the weight of the seeds extracted. Furthermore, the amount of plasma or egg powder or other inhibitors added per well could also be calculated. This gave some indication of the amount of inhibitor needed to decrease HA in the extracts under the given conditions. Such calculation revealed that the proportion plasma/seed meal causing decreased HA of *P sativum* (var Carrera) extracts was 150·6 μg mg^{-1}, while for *V sativa* (var Carole) 10 μg mg^{-1} was calculated. For *Phas vulgaris* the proportion varied between 3·15 mg mg^{-1} and 350·4 μg mg^{-1}, illustrating that as expected, HA and amount of plasma needed for inhibition were closely related. Whether these proportions will also be adequate to inhibit lectin activity in the gastrointestinal tract of piglets or pigs should be investigated in feeding experiments. Fetuin, a specific inhibitor of *Phaseolus* lectin (Pusztai and Watt 1974), caused a larger inhibition of HA than the plasma powders but decreases by addition of mannose to lectins with specificity for this sugar were comparable with effects caused by the plasmas. Table 1 shows that plasma powders caused appreciable inhibition of HA, using Pronase treated erythrocytes, in extracts of *V faba* (var Caspar) and *L angustifolius*. Surprisingly, *L albus* cultivars were not influenced, indicating possible differences in sugar binding properties between lectins of different *Lupinus* sp. HA of *T vulgaris* was also not altered, which was a positive observation in view of future *in vivo* feeding trials with wheat as an important ingredient of the ration. Indeed, it will permit to conclude that, with rations based on wheat and legume seeds, eventual positive effects on animal performance caused by addition of bovine or porcine plasma are not due to interference of the plasma with *T vulgaris* lectin activity. In agree-

ment with earlier work, galactose was a potent inhibitor of lectins in *Lupinus* sp extracts (Kim and Madhusudhan 1988).

As at this stage of the investigation, no other inhibitor than the specific one was found for *L albus* and *Glycine max*, further experiments were carried out with the particular aim of finding appropriate inhibitors for these legume seeds. Duranti *et al* (1996) reported that conglutin γ, a lectin recently isolated and purified from *L albus* seeds, could bind with glycosylated polypeptides, such as ovalbumin. Therefore, the effect of whole egg powder (EP 44) on HA of *L albus*, but also on the other legume seeds, was investigated (Table 1). EP 44 was only a good inhibitor of HA in *L albus* (99·5%) and *Phaseolus* (87·5%) and somewhat less inhibitory on extracts of *L angustifolius*. The former observation could be important concerning isolation and purification of *L albus* lectins using affinity chromatography. Based on the fact that galactose was an inhibitor of *Glycine max* lectin, a series of experiments was done investigating the effect of galactosides (Table 2). As lactose was found to be a powerful inhibitor, the effect of whole or defatted milk powder and whey powder was also determined. Their inhibitory properties are also clearly shown in Table 2.

From the HA experiments, it could be concluded that plasma powders can lower lectin activity in extracts of the legume seeds involved in these experiments, with the exception of *L albus* and *Glycine max*. EP 44 was shown to be an important inhibitor of the former lectin, while HA in extracts of the latter was efficiently decreased by galactosides. As stachyose is known as ANF, its future use as inhibitor (receptor analogue) is not advisable (Dierick and Decuypere 1994). On the other hand, egg powder and milk or whey powder can be used as natural ingredients of rations for pigs.

SDS-PAGE and Western blotting experiments

Gels were loaded with a standard protein mixture and several samples of inhibitors such as bovine and porcine plasma, whole egg powder and fetuin. Gels were stained with Coomassie blue and an example is presented in Fig 1. For bovine plasma (lanes 2 and 7) three important fractions were clearly visible: the albumin band (66 kDa) and the heavy and light chain of immunoglobulin G (IgG; respectively 50 and 25 kDa). In the case of porcine plasma (lanes 3, 4 and 6) the band of the light IgG chain was quantitatively more important and two other small bands were clearly visible (respectively 70 and 45 kDa). Whole egg powder (lane 5) showed a clear ovalbumin band (45 kDa) and in the case of fetuin, the quantitatively most important fraction appeared between 60 and 70 kDa (lane 8). Protein bands on the gels were transferred to nitrocellulose paper and incubated with biotinylated lectins as indicated in legends of Figs 2 and 3. Firstly, there was no binding at all with biotinylated lectins of *Glycine max* and *T vulgaris* (blots not shown). HA series with samples of both lectins

TABLE 2
Inhibition of haemagglutination activity (units mg^{-1} of seeds) in extracts of *Glycine max* by galactosides, dried whole milk powder and whey powder[a]

Inhibitor	*Concentration*	*Var unknown*	*Var Alaric*
None		256–1024	256–1024
Galactose	20 mM	8–64	8–32
Lactose	20 mM	8–64	8–32
Raffinose	20 mM	16–64	16–32
Melibiose	20 mM	8–16	16–32
Stachyose	10 mM	32–64	16–32
Milk powder	1 mg ml^{-1}	512	256
	2 mg ml^{-1}	256–512	128
	10 mg ml^{-1}	8	4
Milk powder (defatted)	1 mg ml^{-1}	512–1024	256
	2 mg ml^{-1}	128	128
	5 mg ml^{-1}	8–16	8
	10 mg ml^{-1}	4	4
Whey powder	1 mg ml^{-1}	64	32
	2 mg ml^{-1}	32	16
	5 mg ml^{-1}	4–8	4–8
	10 mg ml^{-1}	2–4	2–4

[a] All dilution series were done in duplicate and if not identical, both values are given. Experiments with galactosides were repeated twice or three times on different extracts and in this case extreme values are given, also for the blank which explains the sometimes large spread.

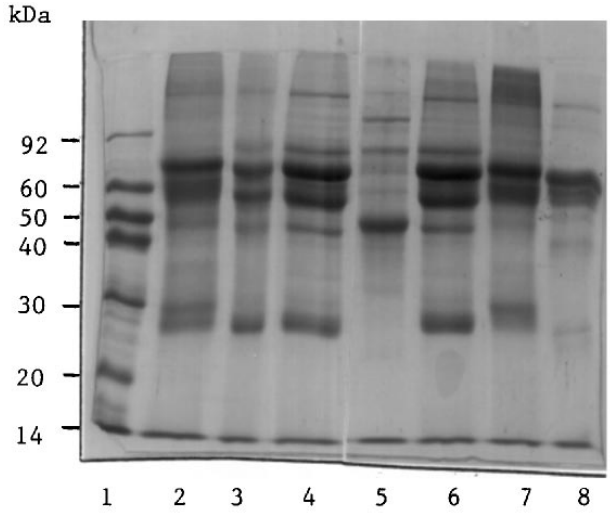

Fig 1. SDS-PAGE of lectin inhibitor preparations stained with Coomassie blue (1) Standard proteins, (2) bovine plasma 1; (3) porcine plasma 1; (4) porcine plasma 2; (5) egg powder 44 EP; (6) porcine plasma 3; (7) bovine plasma 2; (8) fetuin (22·5 μg each loaded).

(Sigma) showed extremely high titres proving that absence of binding was certainly not due to lectin preparations being inactive. These observations were in agreement with the HA experiments showing no effect of several inhibitors on both lectins. *Phaseolus* E lectin bound with all the inhibitors present on the blots and some very interesting phenomena were observed (Fig 2). The albumin fraction of the plasma seemed to be free of lectin, in agreement with the fact that bovine and porcine serum albumin is not glycosylated.

There was also a clear distinction in binding pattern between bovine and porcine plasma, as with the former an intensive binding was seen with glycoproteins having a molecular mass >65 kDa, while the heavy chain of bovine IgG seemed to bind more intensively than

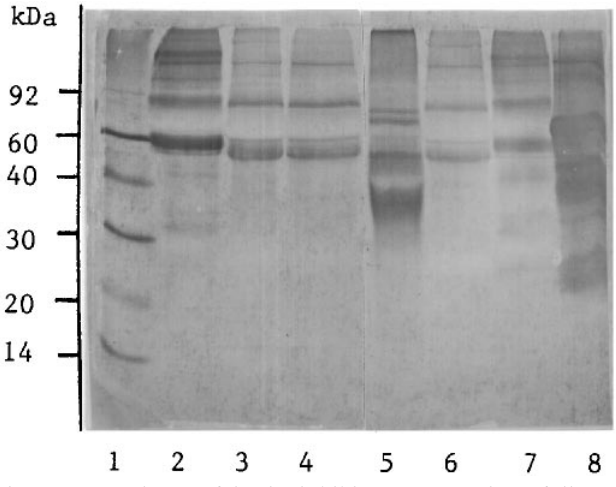

Fig 2. SDS-PAGE of lectin inhibitor preparations followed by blotting and incubation with biotinylated *Phaseolus* E lectin (100 μg). (1)–(8): see Fig 1.

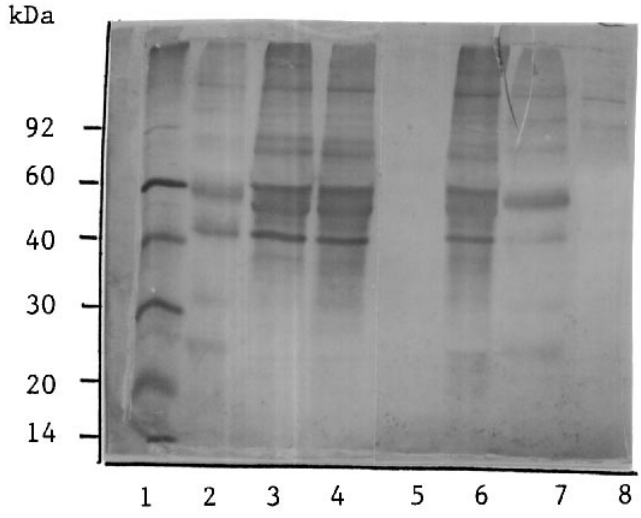

Fig 3. SDS-PAGE of lectin inhibitor preparations followed by blotting and incubation with biotinylated *Pisum sativum* lectin (100 μg). (1)–(8): see Fig 1.

porcine IgG. The light IgG chain showed no lectin binding, indicating that the fraction was not glycosylated, or absence of the specific sugar or oligosaccharide recognisable by the lectins. The lectin also showed good affinity for ovalbumin, while with fetuin extremely intensive binding was seen with glycoproteins between 25 and 100 kDa. It also seemed that the lectin was somewhat more efficiently bound to the bovine than to the porcine plasma samples. Incubations of similar blots with *Phaseolus* L lectin revealed no binding (results not shown) with the blotted inhibitors, due to functional differences in sugar-binding specificities of both isolectins (Miller *et al* 1973; Huisman and Jansman 1991). From the HA experiments with *Phaseolus* extracts and inhibitors described above, no conclusion could be drawn concerning an eventual effect of the plasma powders on isolectin L as this lectin only agglutinates leucocytes. The blotting experiment clearly showed that inhibitors acted by binding with the isolectin E.

A blot incubated with *P sativum* lectin is presented in Fig 3. Again, there seemed to be no binding with plasma albumin and the light IgG chain. The lectin bound more extensively to the porcine plasma completely in agreement with HA data shown in Table 1. Unlike *Phaseolus* E lectin, the *Pisum* lectin also bound to glycoproteins in porcine plasma with molecular mass <45–50 kDa, resulting in a different binding pattern. The lectin showed no affinity towards egg powder, while a high molecular mass fraction of fetuin showed some affinity. HA experiments showed no effect at all of fetuin on *P sativum* lectin (Table 1). Similar blots were incubated with biotinylated lectins of *V sativa*, *V faba* and *L culinaris* (results not shown). Results were comparable with the *Pisum* incubation but a higher amount of lectin (250–500 μg) was needed to obtain a clear picture, possibly due to a lower affinity of the lectins for glycopro-

teins in the inhibitors. The lectins bound also more extensively to porcine plasma.

Finally, identical blots were also incubated with the different lectins in the presence of an excessive amount of specific inhibitor (fetuin for *Phaseolus* lectin, mannose for the others). Only with porcine plasma, a negligible amount of *Pisum* and *Lens* lectin was bound (2–3 very weak and narrow bands at *c* 40–60 kDa). Therefore we can conclude that binding was a specific lectin binding (sugar-protein) and not aspecific (eg protein–protein). Results of SDS-PAGE and Western blotting experiments agreed very well with the HA experiments: lectins which were not inhibited by compounds in the HA experiments showed also no binding with the different protein fractions of the blotted inhibitors. With the exception of *Phaseolus* E lectin, all lectins showed a greater affinity for porcine plasma. As albumin seemed to remain unbound, this fraction could probably be removed from plasma resulting in an increase of the specific inhibitory activity (% of inhibition g^{-1} of plasma). This is an example of the fact that the blotting experiments provided useful additional information, eg concerning the fraction of glycoproteins in inhibitors responsible for their specific action.

In conclusion, it was shown that spray dried plasma powders could inhibit lectin activity in extracts of several legume seeds. Interesting results were also obtained with whole egg powder as far as inhibition of *Lupinus* and *Phaseolus* lectins is concerned. The use of egg powder prepared without yolks could increase the specific inhibitory action as yolks are responsible for the high (44%) lipid content of the powder. Another interesting result was the fact that lectin of *Glycine max* was considerably inhibited by whey powder. Despite the fact that results of this work should be considered as being qualitative rather than quantitative, they permitted clear and unequivocal interpretation. *In vivo* experiments with piglets and fattening pigs will be necessary to investigate whether the inhibitors can neutralise the negative effects of lectin containing rations under the chemical and physico-chemical conditions prevailing in, for example, stomach and duodenum. With this in mind, biotinylated lectins were incubated with blotted plasma powders but in buffers at different pH and it was observed that binding of the lectins to the inhibitors was clearly decreased at pH values $\leqslant 4$ (Van Nevel C J *et al*, to be published).

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