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The influence of trivalent cations and thermal treatment on ruminal degradability of field bean *(Vicia faba)* and rape seed *(Brassica napus)* protein

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ABSTRACT

Treatment with trivalent cations: Fe and Al in the form of alums (2–40 g of metal/1 kg of feed) as a means of reducing degradation of field bean (FB) and rape seed (RS) protein in the rumen was studied using solubility tests and *in sacco* degradation as response criteria. The chemical treatments were also combined with autoclaving at 121°C for 10–30 min. and wet heating at 120°C for 20 and 30 min. The results were compared to the effects of formaldehyde.

Iron alum was as effective as formaldehyde in reducing protein solubility of FB ($P > 0.05$) and more active than aluminium alum ($P < 0.01$). FB was more responsive to Fe than RS and reacted to increasing Fe concentrations up to 40 g/kg. In the range of 20–40 g Fe/kg, FB protein degradation was reduced by 34 to 51 units, respectively, that of RS by 26 to 31 units.

Autoclaving in combination with Fe action was particularly effective in reducing ruminal degradability of FB ($P < 0.001$). The amount of undegraded protein from treated samples was 3–5 times higher than that in the non-autoclaved water-treated samples. The effect of autoclaving was much less pronounced on RS ($P < 0.05$), as was wet heating at atmospheric pressure ($P > 0.05$).

KEY WORDS: protein protection, trivalent cations, formaldehyde, field bean, rape seed

INTRODUCTION

Feed protein is degraded by rumen microbes to amino acids and ammonia and these products may be incorporated into microbial protein. Undegraded alimentary protein, that is available for digestion and absorption form the small intestine, together with microbial protein contribute to the protein supply to the host animal.

Susceptibility of various feedstuffs to ruminal degradation ranges from 30 to 95%, however for a majority of common protein sources such as legume and oil seeds this value nears 90% (Antoniewicz et al., 1984; de Boer et al., 1987; Van Straalen and Tamminga, 1990; Antoniewicz et al., 1992 a, b).

Processing of concentrate feedstuffs aimed at reducing ruminal protein degradation results in increasing the amount of bypass protein flowing to the duodenum. This can be beneficial to an animal only when the protein escaping ruminal degradation is digestible further on in the small intestine. Wet and dry heating at normal or increased pressure was found effective in reducing protein degradability (Chrenkova et al., 1986; Skraba and Antoniewicz, 1986; Stern et al., 1985; Vanbelle et al., 1989). However, excessive treatment may result in depressed postruminal protein availability (Satter, 1986).

Chemical treatments are based on specific reactions between reactive side groups in proteins and aldehydes or natural tannins. Although studies of these methods began in the 1960's, they have been still continued (Driedger and Hatfield, 1972; Schmidt et al., 1973; Nishimuta et al., 1974; Makkar et al., 1988). Formaldehyde was widely used in experiments both in the laboratory and on animals (Vérité et al., 1977, Thomas et al., 1979; Phillips, 1981; Antoniewicz et al., 1987; Kowalczyk, 1991).

However, because of its harmful effect on human health, formaldehyde has not been approved as a feed additive in many countries, including Poland. That is why alternative reagents, such as acetid acid (Vicini et al., 1983), alcohols (Lynch et al., 1987, 1988; van der Aar et al., 1982), xylose and glucose (Cleale et al., 1987 a, b) and their combinations with heat and pressure treatments (van der Aar et al., 1984) were studied to assess their ability to reduce ruminal degradation of protein.

The use of salts of polyvalent cations capable of binding to protein seemed to be plausible method of reducing feed protein degradability in the rumen. The purpose of our study was to evaluate the suitability of salts of trivalent iron and aluminium cations to reduce ruminal degradation of protein. Their efficacy was compared with that of formaldehyde.

MATERIALS AND METHODS

Feedstuffs

The efficacy of chemical agents was evaluated using ground field bean *(Vicia faba)* (FB) and rape *(Brassica napus)* (RS) seeds. The crude protein content of the feeds was 280 and 251 g per kg of DM, respectively. The feeds differed markedly in their protein: fat ratio which was 37:1 in FB and 1:2 in RS. FB was ground to pass a 1 mm screen and RS was hammer-milled so that it was crushed and halved.

Treatment of feeds with various agents

1. Treatment with formaldehyde

Samples of 100 g kept in polythene bags were treated with water or aqueous

solutions of formaldehyde (made by dilution of formalin) at volumes ensuring a 50% final total moisture content of the sample (Antoniewicz et al., 1992 a). The solutions contained formaldehyde in such amounts that its final concentration equalled 2, 4, 6, and 8 g kg^{-1} in air-dried feed. The material was thoroughly mixed, left for 24 h at room temperature and then oven dried at 60°C overnight.

2. Treatment with Al^{3+} and Fe^{3+} salts

Water soluble salts: aluminium ammonium sulphate ($Al(NH_4)(SO_4)_2 \cdot 12 \cdot H_2O$, analytical grade, POCh Gliwice, Poland) and iron ammonium sulphate ($Fe(NH_4)(SO_4)_2 \cdot 12\ H_2O$, analytical grade, POCh Gliwice, Poland) were used.

The treatment process was conducted similarly as with formaldehyde, except that the final metal concentration was 2–10 g Al and 2–40 g Fe per 1 kg of treated feed. Initial comparison of the efficacy of Al and Fe salts was done using FB only.

3. Fe treatment followed by autoclaving or oven heating

Feed samples were treated with water or iron alum solutions (4, 8 or 12 g Fe kg^{-1} of sample) at 50% moisture, as described for formaldehyde treatment. The thoroughly mixed wet cakes of feeds were placed on trays and autoclaved at 121°C for 10, 20 or 30 min. Trays with other portions of similarly prepared samples were tightly covered with aluminum foil and heated in an oven at 120°C for 20 or 30 min. The samples were left for 2 h at room temperature and then oven-dried at 60°C overnight.

The samples of field beans treated with water or salt solutions to a final concentration of up to 3 g of metal per kg of feed, tended to form firm, sticky cakes, which were coarsly ground (KT-30 grinder, Falling Number, Sweden, with the most loose set of disks) after drying.

The efficacy of protein protection was evaluated by determining its solubility in 0.15 M NaCl (Miller, 1982), susceptibility to papain as described by Maciejewicz-Ryś (1979) and dry matter and protein disappearance during *in sacco* incubations in sheep rumen (Antoniewicz et al., 1984). Digestion with pepsin and pancreatin (Akeson and Stahman, 1964; Antoniewicz et al., 1992 b) was used to test the reversibility of the processing methods applied.

Solubility test

Triplicate samples of 0.5 g of processed feeds were shaken with 50 ml of 0.15 M NaCl at room temperature for 2 h. Feed residue was filtered through ash-free filter paper (Filtrak, Niederschlag, Germany), washed with distilled water untill no chloride ions were found in the effluate and the protein content in the residue was determined.

Digestion with papain

Triplicate samples of 0.5 g of processed feeds were shaken with 2 ml of 4% aqueous solution of papain (1:350, Loba-Chemie, Wien-Fischamend, Austria) and 20 ml of citrate buffer pH 7.2 (5 g of sodium citrate and 30 mg NaCN dissolved in bidistilled water, pH adjusted to 7.2 with 0.3 M H_3PO_4 and the solution made up to 1000 ml with bidistilled water) in a water bath at 56°C for 3 h. The residue was filtered through ash-free filter paper, washed four times with warm distilled water and wet-ashed with filter for Kjeldahl N determination. The results wers corrected against reagent (enzyme + filter) blank run through the whole procedure.

Digestion with pepsin and pancreatin

Triplicate samples of 0.5 g of processed feeds were shaken with 20 ml of a pepsin (1:10 000, Sigma, USA) solution (2 g l^{-1}) in 0.075 M HCl in a water bath at 39° C for 3 h. The pH of the liquid was then increased to 7 by addition of 0.9 ml of 1 M Na_2CO_3. Next, 20 ml of a pancreatin (Polfa, Warszawa, 59U of trypsin g^{-1}) solution (2 g l^{-1}) in 0.1 M phosphate buffer pH 7.4 (80 ml 0.2 M NaH_2PO_4 + 420 ml 0.2 M Na_2HPO_4 made up to 1 l with distilled water) were added and incubation was continued with shaking in a water bath at 39°C for 2 h. Another 20 ml of buffer (39°C) was then added and the incubation mixture was left overnight at 39°C without shaking. The solid residue was filtered through ash-free filter paper and treated similarly as after incubation with papain.

*Incubations **in sacco***

Nylon bags (9 × 15 cm, 40 μm pore size) were prepared as described by Antoniewicz et al. (1984) and filled with 5 g of material, each sample in six replicates. The bags (six at a time) were placed in the rumen of 4 mature (body weight av. 60 kg) wethers fitted with rumen cannula 3 cm in diameter, for 17 h. The wethers received a maintenance ration of 1000 g meadow hay and 200 g ground barley (9.5 MJ ME/d) given in two equal meals at 7.00 (after removing the bags) and 13.00 h. The bags, after removing, were thoroughly washed with lukewarm tap water for 15 min. and then freeze-dried. The weight of the residue was determined and aliquots were analysed for dry matter and protein content.

Chemical and statistical analyses

Dry matter content was estimated by oven drying at 100°C for 3 h and weighing after cooling in a dessicator. Nitrogen was determined by Kjeldahl

analysis using a Kjeltec Auto 1030 analyser (Tecator, Sweden). Protein contents and degradability values determined in the samples treated with alums were corrected for the amounts of ammonia introduced by the processing. Ammonia was considered completely soluble and its amount was substracted from the original N content of sample (weighed for extraction or nylon bag incubation).

The results were subjected to simple and two-factorial analysis of variance. Treatment means were compared using a multiple F-test.

RESULTS

Comparison of the protective effect of aluminium and iron alums

Water-treated FB protein had a solubility of about 0.33 in 0.15 M NaCl (Table 1). Treatment with formaldehyde or trivalent ions reduced this value to 0.07–0.20 (by 80 to 50%, $P < 0.001$). Iron ammonium sulphate was almost as effective as formaldehyde in reducing solubility ($P > 0.05$) and more active than aluminium alum ($P < 0.01$). On incubation with papain, the solubilization of protein of processed FB ranked similarly as in the 0.15 M NaCl extracts, again proving the ability of iron alum to lower protein solubility (Table 1). The reactions

TABLE 1

Protein solubility in 0.15 M NaCl and susceptibility to papain. Ground field beans were treated with either aluminium or iron alums or formaldehyde (mean values over 3 replicates)

Concetration of chemical agent (g/kg of feed)	Aluminium alum	Iron alum	Formaldehyde
		Extration with 0.15 M NaCl	
0	0.34	0.34	0.33
2	0.25	0.19	0.30
4	0.20	0.15	0.18
6	0.19	0.07	0.10
8	0.19	0.07	0.08
SEM	0.003	0.002	0.003
		Digestion with papain	
0	0.46	0.45	0.46
2	0.37	0.31	0.38
4	0.32	0.20	0.30
6	0.30	0.16	0.16
8	0.29	0.13	0.16
SEM	0.003	0.004	0.004

TABLE 2

The effect of formaldehyde treatment of field beans (FB) and rape seeds (RS) on their susceptibility to protein degradation during 17 h *in sacco* incubation in the rumen of sheep (RD) and to pepsin-pancreatin digestion (PPS)

Formaldehyde concentration (g/kg of feed)	RD[e]		Level of significance of differences (FB vs. RS	PPS[f]	
	FB	RS		FB	RS
0	0.89 Aa	0.90 Aa	NS	0.95	0.92
2	0.81 Aab	0.78 AA	NS	0.91	0.90
4	0.75 Ab	0.57 Bb	**	0.90	0.86
6	0.60 Cc	0.43 Cc	**	0.90	0.83
8	0.35 Dd	0.41 Cc	NS	0.87	0.83
SEM	0.007	0.011		0.004	0.004

[e] mean values over 6 replicates
[f] mean values over 3 replicates
Means in the same column marked with various letters are significantly different; capitals denote $P < 0.01$, small letters $P < 0.05$

of FB protein with trivalent cations were fully reversible by pepsin and pancreatin (Table 2).

Effect of formaldehyde treatment of FB and RS on in sacco disppearance and pepsin-pancreatin solubility of protein

Formaldehyde at concentrations from 4 to 8 g/kg caused a respective reduction of *in sacco* protein disappearance by 14 to 54 units ($P < 0.001$) in FB and by 33 to 49 units ($P < 0.01$) in RS. Digestibility in the pepsin-pancreatin system was reduced by 5–8 units in FB and 6–9 units in RS, respectively (Table 2).

Effect of iron alum treatment at room temperature

Incubations *in sacco* in sheep rumen showed (Table 3) that the main effect of Fe at concentrations up to 10 g/kg of feed was protection of soluble proteins. At 10 g Fe/kg , 79% of FB and RS protein was still degradable. A substantial decrease of FB and RS protein degradability was found at 20 g Fe/kg, 0.55 and 0.65, respectively. Further increases of Fe concentrations to 40 g/kg feed had a more pronounced effect on FB protein degradability as compared with RS ($P < 0.01$). When the Fe concentration increased from 20 to 40 g/kg, protein disappearance in the rumen was reduced from 0.55 to 0.38 in FB and from 0.64 to 0.59 in RS, respectively (Table 3). There was 3.8 (RS) and 5.4 (FB) times more

TABLE 3

The effect of iron alum treatment of field beans (FB) and rape seeds (RS) on their susceptibility to protein degradation during 17 h incubation in the rumen of sheep (RD) and to pepsin-pancreatin digestion (PPS)

FE concentration (g/kg of feed)	RD[e]		Level of significance of differences (FB vs. RS)	PPS[f]	
	FB	RS		FB	RS
0	0.89 Aa	0.90 Aa	NS	0.88	0.85
5	0.87 Aa	0.85 Aa	NS	0.88	0.85
10	0.78 Aa	0.79 Aa	NS	0.86	0.84
20	0.55 Bb	0.64 Bb	*	0.86	0.82
30	0.41 BCc	0.62 Bb	**	0.86	0.82
40	0.38 Cc	0.59 Bb	**	0.86	0.82
SEM	0.006	0.009		0.004	0.005

[e] mean values over 6 replicates
[f] mean values over 3 replicates
Means in the same column marked with various letters are significantly different; capitals denote $P < 0.01$, small letters $P < 0.05$

TABLE 4

The effects of iron alum treatment and autoclaving at 121 °C of field beans (FB) and rape seeds (RS) on protein disappearance during *in sacco* incubation in the rumen of sheep (data from two-factor analysis of variance)

Factor	FB		RS	
	Mean	SEM	Mean	SEM
Fe concetration (g/kg of feed)				
0	0.64[e]A	0.035	0.83[e]A	0.013
4	0.60 A	0.036	0.84 A	0.011
8	0.47 B	0.040	0.69 B	0.018
12	0.48 B	0.030	0.64 B	0.017
Autoclaving time (min)				
0	0.82[f]Aa	0.021	0.81[f]a	0.024
10	0.52 Bb	0.023	0.74 ab	0.024
20	0.46 Bc	0.019	0.73 ab	0.019
30	0.39 Cd	0.017	0.70 b	0.022
Interaction	$P = 0.03$		$P > 0.05$	

[e] mean values over 4 autoclaving times, 6 replicates per each treatment ($n = 24$)
[f] mean values over 4 Fe concentrations, 6 replicates per each treatment ($n = 24$)
The means within the same column and treatment marked with various letters are significantly different; capitals denote $P < 0.01$, small letters $P < 0.05$

non-degradable protein in the residue from 30 g Fe/kg than from water-treated samples (Fig. 1). The decrease in protein digestibility in the pepsin-pancreatin system was only reduced by 2-3 percent when the Fe concentration exceeded 10g/kg (Table 3).

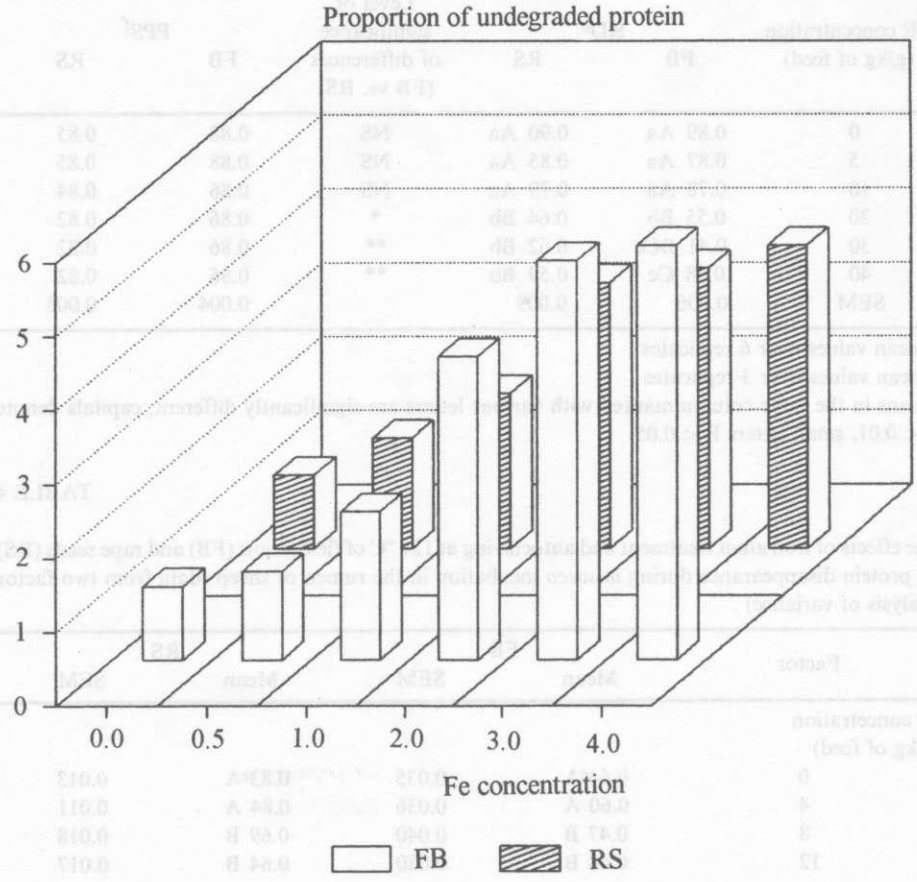

Figure 1. Proportion of undegraded protein (UP) after 17 h *in sacco* incubation in sheep rumen of field beans (FB) and rape seed (RS) treated with increasing amounts of Fe (%, w/w). Amount left from water-treated sample was considered as 1

Effect of iron alum and thermal treatments

Autoclaving was very effective in reducing ruminal degradability of FB protein ($P < 0.001$; Table 4). The amount of undegraded protein left in the nylon bag after incubation in the rumen was 3–5 times higher than in the non-autoclaved water-treated sample (Fig. 2). The effect of autoclaving was

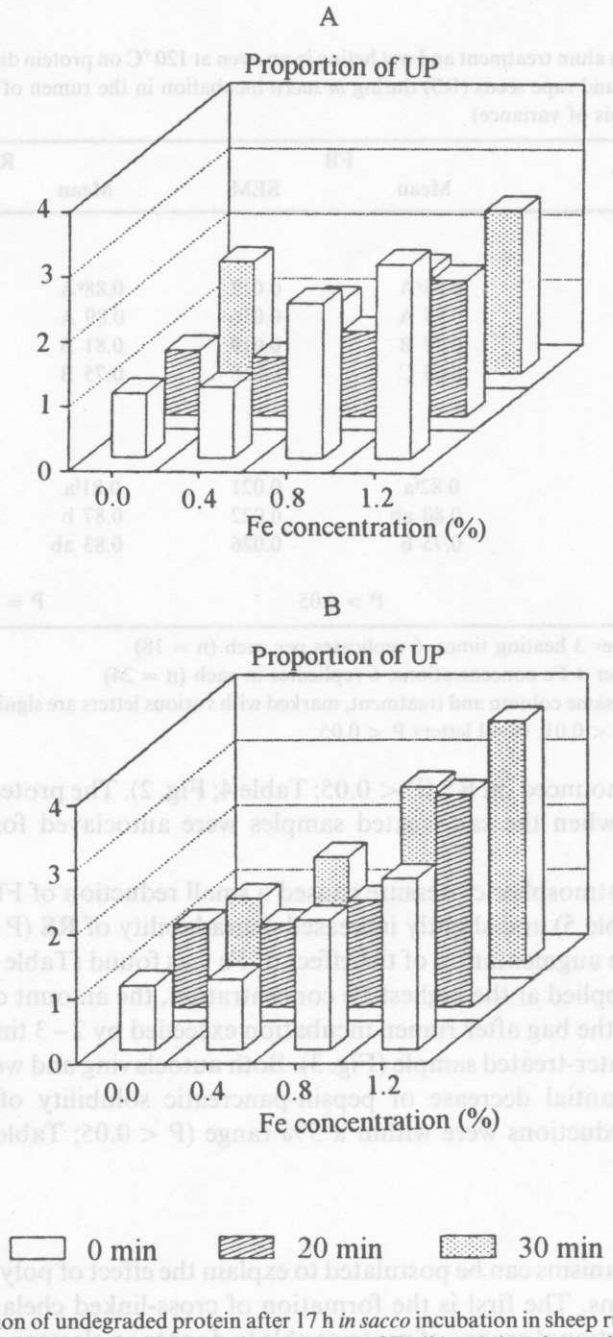

Figure 2. Proportion of undegraded protein after 17 h *in sacco* incubation in sheep rumen of rape seed (A) and field beans (B) treated with increasing amounts of Fe (%, w/w) and autoclaved at 121°C for 10-30 min. Amount left from water-treated non-autoclaved samples was considered as 1

TABLE 5

The effects of iron alum treatment and wet heting in an oven at 120 °C on protein disappearance from field beans (FB) and rape seeds (RS) during *in sacco* incubation in the rumen of sheep (data from two-factor analysis of variance)

Factor	FB		RS	
	Mean	SEM	Mean	SEM
Fe concetration (g/kg of feed)				
0	0.88[e]A	0.012	0.88[e]A	0.012
4	0.85 A	0.016	0.89 A	0.013
8	0.77 B	0.019	0.81 B	0.021
12	0.65 C	0.018	0.75 B	0.022
Heating time (min)				
0	0.82[f]a	0.021	0.81[f]a	0.024
20	0.80 ab	0.022	0.87 b	0.016
30	0.75 b	0.026	0.83 ab	0.016
Interaction	$P > 0.05$		$P = 0.02$	

[e] Mean values over 3 heating times, 6 replicates per each (n = 18)
[f] Mean values over 4 Fe concentrations, 6 replicates in each (n = 24)
Means within the same column and treatment, marked with various letters are significantly different; capitals denote $P < 0.01$, small letters $P < 0.05$

much less pronounced on RS ($P < 0.05$; Table 4; Fig. 2). The protective ability of Fe increased when the salt-treated samples were autoclaved for 10–30 min. (Table 4).

Heating at atmospheric pressure caused a small reduction of FB degradation ($P < 0.05$; Table 5) and slightly increased degradability of RS ($P < 0.05$; Table 5). No positive augmentation of the effect of Fe was found (Table 5). Still, when heating was applied at the highest Fe concentration, the amount of undegraded protein left in the bag after rumen incubation exceeded by 2–3 times that in the non-heated water-treated sample (Fig. 3). Both autoclaving and wet heat did not cause a substantial decrease of pepsin-pancreatic solubility of FB and RS protein; the reductions were within a 5% range ($P < 0.05$; Table 6).

DISCUSSION

Three mechanisms can be postulated to explain the effect of polyvalent cations on feed proteins. The first is the formation of cross-linked chelate-type bonds with those functional groups of proteins able to donate an electron pair (Kincaid, 1989; Thompson and Fowler, 1990). The second is to interfere with the micellar

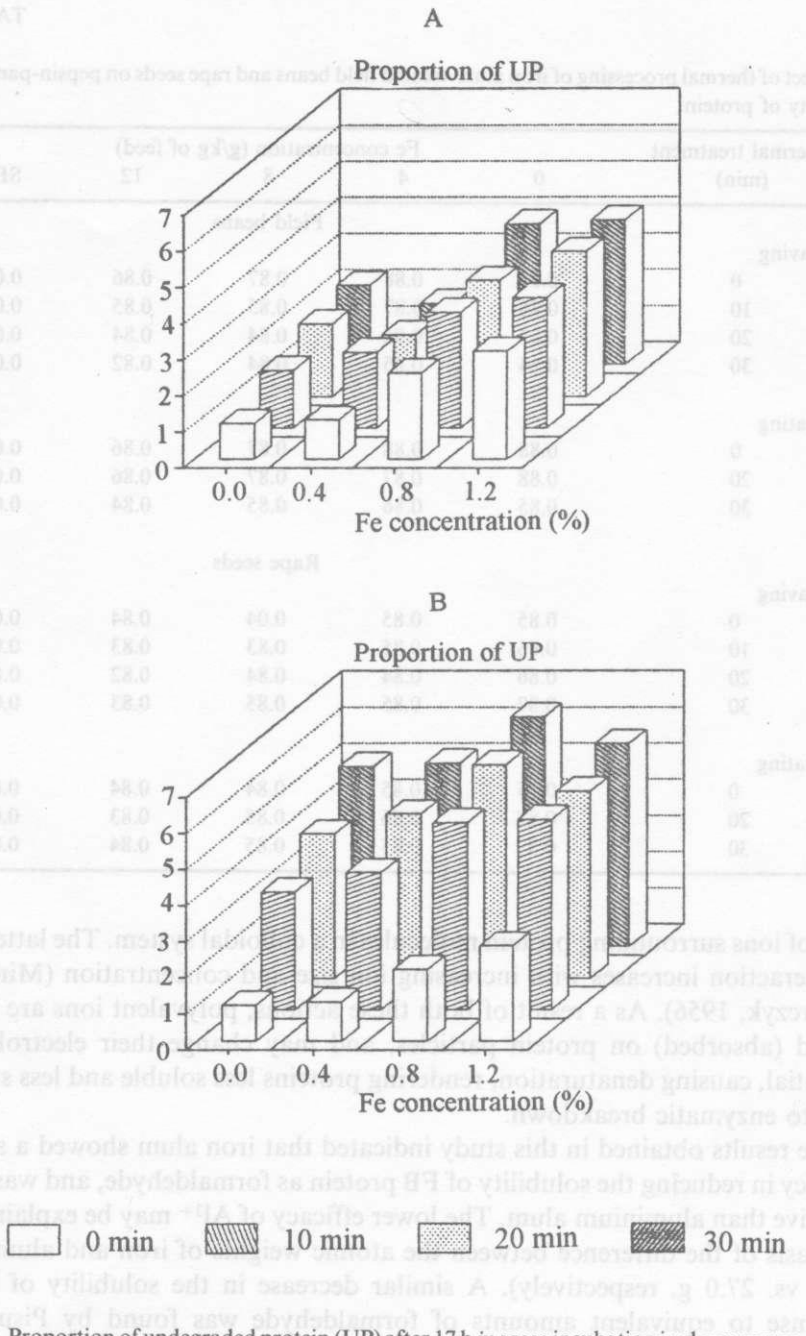

Figure 3. Proportion of undegraded protein (UP) after 17 h *in sacco* incubation in sheep rumen of rape seed (A) and field beans (B) treated with increasing amounts of Fe (%, w/w) and wet-heated at 120°C for 20 or 30 min. Amount left from water-treated, not heated samples was considered as 1

TABLE 6

The effect of thermal processing of iron alum-treated field beans and rape seeds on pepsin-pancreatin solubility of protein

Thermal treatment (min)	Fe concentration (g/kg of feed) 0	4	8	12	SEM
			Field beans		
Autoclaving					
0	0.88	0.88	0.87	0.86	0.006
10	0.86	0.87	0.85	0.85	0.007
20	0.85	0.86	0.84	0.84	0.008
30	0.84	0.85	0.84	0.82	0.011
Wet heating					
0	0.88	0.88	0.87	0.86	0.007
20	0.88	0.87	0.87	0.86	0.011
30	0.85	0.86	0.85	0.84	0.009
			Rape seeds		
Autoclaving					
0	0.85	0.85	0.04	0.84	0.009
10	0.86	0.85	0.83	0.83	0.008
20	0.86	0.84	0.84	0.82	0.009
30	0.87	0.85	0.85	0.83	0.010
Wet heating					
0	0.85	0.85	0.84	0.84	0.009
20	0.87	0.86	0.85	0.83	0.010
30	0.87	0.85	0.85	0.84	0.011

zone of ions surrounding protein molecules in a colloidal system. The latter type of interaction increases with increasing ion size and concentration (Minc and Stolarczyk, 1956). As a result of both these actions, polyvalent ions are firmly bound (absorbed) on protein particles, and may change their electrokinetic potential, causing denaturation, rendering proteins less soluble and less susceptible to enzymatic breakdown.

The results obtained in this study indicated that iron alum showed a similar efficacy in reducing the solubility of FB protein as formaldehyde, and was more effective than aluminium alum. The lower efficacy of Al^{3+} may be explained on the basis of the difference between the atomic weights of iron and aluminium (55.9 vs. 27.0 g, respectively). A similar decrease in the solubility of FB in response to equivalent amounts of formaldehyde was found by Pisulewski (1975).

The third possible mechanism is the binding of polyvalent cations to phytates

present in relatively high levels in RS and FB (7.3 and 4.7 g phytic P/kg, respectively). It is well known that ferric ions are particularly reactive and they are used as a precipitating agent in the quantitative determination of phytates (Antoniewicz et al., 1992).

In our study, marked differences between the susceptibility of RS and FB to chemical and physical agents were found. They can be related to the differences in the composition of the seeds. Much lower susceptibility of RS to ferric ions may result from the 45% oil content of these seeds. Thus, iron may be utilized to form soaps with fatty acids. Also, non-polar fatty acid chains can make the access of ions to protein molecules more difficult. The latter explanation seems more probable since the efficacy of protein protection was not substantially improved with increasing Fe^{3+} concentration. However, in our earlier study (Antoniewicz et al., 1992a), no adverse effect of fat on protection of RS with formaldehyde was found.

In sacco degradability of FB protein was strongly reduced by autoclaving. This effect could have been attributed to a relatively high tannin content in this material (4.5 g/kg; Ernest, 1987). Decreased ruminal protein digestibility of tannin-containing FB following autoclaving, although smaller than in the present study, was found by Buckley et al. (1983).

All of the treatments used in this study were almost entirely reversible by pepsin-pancreatin, which indicates that the bonds formed between seed components and trivalent iron are dissociable under mild acidic conditions. The enzymatic method used to determine intestinal digestibility of protected proteins is reliable because it gives results well correlated with the digestibility and quality of feed protein *in vivo* (Akeson and Stahmann, 1964) and with a mobile bag technique (Antoniewicz et al., 1992b).

Without thermal treatment, 20 g Fe/kg of feed (RS and FB) were necessary to reduce protein degradability by 23–35 units, equivalent to the protective action of about 5 g of formaldehyde/kg. With autoclaving, 4–8 g of Fe/kg of FB led to the same results.

One should consider the nutritional consequences of feeding high dietary Fe levels when using iron alums to reduce protein degradation. Rose et al. (1982) found that 760 ppm of Fe in a diet for growing lambs caused significant depression of feed intake and daily gain ($P < 0.01$) and increased deposition of iron in liver, kidney and spleen, without any effect on bone mineralization. Iron absorption from the alimentary tract is related to the body needs and is more efficient in young than in mature animals (Bondi, 1987). A maximum safe level of iron for the lactating dairy cow is 1000 mg/kg of ration (Thompson and Fowler, 1990). This means that if 1 kg of protected feeds is to be included in a daily ration of 15 kg of DM, the highest permissible level of Fe used to protect the seed protein would be 15 g/kg of the feed. If chemical treatment was combined with

autoclaving, the amount of Fe needed would be reduced by 50% and the amount of protected FB seeds in a daily ration could be increased two-fold.

One of the more important negative effects of Fe surplus may be poorer phosphorus utilization; higher dietary Fe doses may cause an increased phosphorus requirement.

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STRESZCZENIE

Wpływ działania trójwartościowych kationów i podniesionej temperatury na podatność na rozkład w żwaczu białka nasion bobiku *(Vicia faba)* i **rzepaku** *(Brassica napus)*

Badano działanie kationów trójwartościowych żelaza i glinu podawanych jako ałun żelazowy i glinowy w ilości 2–40 g metalu na 1 kg paszy, na rozkład w żwaczu białka nasion bobiku (FB)

i rzepaku (RS). Jako wskaźniki zastosowano test rozpuszczalności i rozkład białka przy inkubacji w woreczkach nylonowych w żwaczu. Działanie chemiczne łączono także z autoklawowaniem w 121°C przez 10–30 min. i ogrzewaniem zwilżonych nasion w 120°C przez 20 i 30 min. Kontrolę stanowiły próby nasion poddane działaniu formaldehydu.

Działanie ałunem żelazowym powodowało podobne zmniejszanie rozpuszczalności białka FB jak działanie formaldehydem ($P < 0.05$). Ałun glinowy był mniej efektywny ($P < 0.01$). FB wykazywał większą podatność na działanie Fe niż RS i reagował na zwiększenie stężenia Fe do 40 g/kg. Przy działaniu 20–40 g Fe/kg, rozkład białka FB w żwaczu ulegał zmniejszeniu o 34 do 51 jednostek, a rozkład rzepaku, odpowiednio, o 26 do 31 jednostek.

Autoklawowanie w połączeniu z działaniem Fe powodowało znaczne zmniejszenie rozkładu w żwaczu białka FB ($P < 0.001$). Ilość nie rozłożonego białka, pozostająca po inkubacji, była 3–5 razy większa niż przy trawieniu kontrolnych próbek nie autoklawowanych i traktowanych jedynie wodą. Białko RS było znacznie mniej podatne na autoklawowanie i ogrzewanie wilgotnych prób przy ciśnieniu atmosferycznym; istotność różnic w degradacji białka w stosunku do próbek nie poddanych ogrzewaniu wynosiła odpowiednio $P < 0.05$ i $P > 0.05$.

Działanie Fe i podniesionej temperatury nie powodowały istotnego zmniejszenia rozpuszczalności białek pod działaniem pepsyny i pankreatyny ($P > 0.05$).