Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal

Woong-Yeoul Park[1], Tohru Matsui[1]*, Chiaki Konishi[1], Sung-Won Kim[1], Fumiko Yano[2] and Hideo Yano[1]

[1]*Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502 Japan*
[2]*Faculty of Biology-Oriented Science, Kinki University, Uchita, Wakayama, Japan*

(Received 29 June 1998 – Revised 20 November 1998 – Accepted 10 February 1999)

Most of the P in oilseed meal is in the form of phytate P, and phytate forms complexes with protein. Phytate P has been considered to be absorbed easily in ruminants because of phytate degradation in the rumen. Treatment of oilseed meals with formaldehyde improves the nutritional value of protein through suppressing its ruminal degradation. The present experiment was conducted to study the effects of formaldehyde treatment on phytate degradation in the rumen. The ruminal degradation of phytate in formaldehyde-treated soyabean meal or rapeseed meal was determined by a nylon-bag technique in sheep. Soyabean meal and rapeseed meal were treated with formaldehyde at levels of 3, 5 or 10 g/kg. Treatment with formaldehyde suppressed phytate and protein degradation in both the oilseed meals. Compared with the regular soyabean meal, the regular rapeseed meal showed lower degradability of phytate in the rumen. These results suggest that treatment with formaldehyde suppresses ruminal degradation of phytate in oilseed meal. Thus, the absorption of P from oilseed meal is probably decreased by this treatment in ruminants.

Phytate phosphorus: Oilseed meal: Formaldehyde treatment: Sheep

The large amount of P in animal waste is a concern in areas where livestock population density is high (Cromewell & Coffey, 1991). In single-stomached animals and poultry, P in oilseed meals is poorly utilized because most is present in the form of phytate P which is not easily absorbed (Reddy *et al*. 1982). Supplementation with microbial phytase (*EC* 3.1.3.8) improved the utilization of phytate P in these animals (Simons *et al*. 1990). On the other hand, phytate P has been considered to be utilized efficiently in ruminants. Nelson *et al*. (1976) showed that phytate was not found in the rumen, abomasum or intestine of calves fed on a soyabean-meal-based diet, and they suggested that phytate was completely hydrolysed in the rumen. Ellis & Tillman (1961) reported that wethers could absorb appreciable amounts of the phytate P in wheat bran.

Soyabean meal and rapeseed meal are common protein sources in ruminant feed. Many studies have been performed to decrease the ruminal degradability of protein in these oilseed meals using formaldehyde treatment because suppression of protein degradation in the rumen improves protein utilization (Nishimuta *et al*. 1974; Spears *et al*. 1980; Coombe, 1985). The decrease in protein degradation is considered to be the result of lowering the solubility of protein in the rumen (Nishimuta *et al*. 1974). Thomas *et al*. (1946) reported that phytate in natural feed formed stable complexes with protein. Therefore, the reduction of ruminal degradability of protein may suppress the degradation of phytate in the rumen.

The objective of the present experiment was to determine the influence of formaldehyde treatment on ruminal degradation of phytate in soyabean meal and rapeseed meal using a nylon-bag technique in sheep.

Materials and methods

Formaldehyde treatment

Reagent grade formaldehyde was added to soyabean meal and rapeseed meal at levels of 3 (F0·3), 5 (F0·5) or 10 (F1·0) g/kg on an air-dry matter basis. After addition of formaldehyde solution, the meal was mixed well, poured into plastic bags and sealed for 3 d to prevent volatilization of formaldehyde. Then, the treated meal was poured onto a plastic sheet to remove formaldehyde. These treatments were replicated three times. The three batches of treated oilseed meal were mixed well for the *in sacco* incubation study.

Abbreviations: ED, effective degradability; F0·3, F0·5 and F1·0, treatment with 3, 5 and 10 g formaldehyde/kg meal respectively; PCR, the ratio phytate phosphorus : crude protein in their effective degradabilities.

***Corresponding author:** Dr Tohru Matsui, fax +81 75 753 6344, email matsui@jkans.jkans.kais.kyoto-u.ac.jp

Animals and feeding

Three crossbred sheep (Suffolk × Corriedale) weighing approximately 40 kg, each fitted with a rumen cannula, were used. The sheep were individually housed in metabolism cages and cared for according to the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee). The diet consisted of (g/kg): 100 soyabean meal, 300 barley grain and 600 timothy (*Phleum pratense*) hay. The animals were fed twice daily (10.00 and 22.00 hours) at a level of 8·5 g/kg body weight with free access to water and mineral block. After a 1-week preliminary period, sheep were used for the incubation trial.

Incubation of soyabean meal and rapeseed meal in sacco

Regular and treated soyabean meal and rapeseed meal were ground to pass through a mesh screen with a pore size of 2 mm. Approximately 5 g each ground sample was poured into a nylon bag (40 × 165 mm: pore size, 900 μm^2). Six bags, each containing regular or treated oilseed meal, were introduced into the rumen through the ruminal cannula just after the morning feed and incubated in the rumen. After 3, 6, 12, 24, 36 or 48 h incubation in the rumen, the nylon bags were recovered. Each type of oilseed meal was incubated in the rumen of sheep in duplicate. The bags were rinsed thoroughly with running tap water until the wash-out water became clear. The nylon bag from 0 h incubation for each sample was not incubated in the rumen and only rinsed. The residues in the bags were lyophilized.

Chemical analyses

Phytate P and crude protein were analysed in ground samples and the residue after rinsing. Phytate P was determined according to the method of the Association of Official Analytical Chemists (1990). Kjeldahl N was determined and crude protein was calculated by multiplication of N by 6·25.

Calculation of effective degradability

The relationship between disappearance of phytate P or crude protein from nylon bags (Y) and the incubation time (X) was described by the model proposed by Ørskov & McDonald (1979):

$$Y = A + B(1 - e^{-CX)},$$

where A, B and C are constants fitted by a least-squares procedure to estimate the rapidly soluble fraction (A), the degradable fraction (B) and the rate constant (C) for the degradation of B respectively. The ruminal effective degradability (ED) of phytate P or crude protein was calculated by the constants A, B and C and the three different ruminal-passage rates, i.e. 0·02, 0·05 and 0·08/h. These calculations were carried out using the 'Neway' computer program (X. B. Chen, Rowett Research Institute, Aberdeen, UK) based on the models of Ørskov & McDonald (1979) and McDonald (1981).

Table 1. Crude protein and phytate phosphorus concentrations (g/kg) in regular and treated oilseed meals

(Mean values with their standard errors for three replicated treatments of the same batch of oilseed meal)

	Crude protein	Phytate phosphorus
Soyabean meal		
Regular	564	4·45
F0·3	569	4·46
F0·5	562	4·35
F1·0	569	4·42
SEM	4	0·02
Rapeseed meal		
Regular	412	9·84
F0·3	403	9·64
F0·5	404	9·60
F1·0	413	9·57
SEM	6	0·21

F0·3, F0·5 and F1·0, oilseed meals treated with formaldehyde at levels of 3, 5 and 10 g/kg respectively.

Statistical analysis

The mean of each duplicate incubation in an individual sheep served as the experimental unit. Data were analysed by two-way ANOVA. For comparison among the treatments, the least-significant difference test was used. Differences were considered significant at $P < 0·05$. All statistical analyses were performed with the general linear models procedure of the SAS program (1985; Statistical Analysis Systems, Cary, NC, USA).

Results

The concentration of phytate P in the regular rapeseed meal was twice that in the regular soyabean meal (Table 1). Formaldehyde treatment did not affect phytate P or crude protein concentration in either oilseed meal.

The rapidly soluble fraction (A) of crude protein was significantly lower ($P < 0·05$) in the soyabean meals treated with F0·5 and F1·0 compared with the regular soyabean meal, and these treatments significantly ($P < 0·05$) decreased A of crude protein in rapeseed (Table 2). Although the regular soyabean meal showed a significantly ($P < 0·05$) lower A value than the regular rapeseed meal, the difference between the oilseed meals disappeared after formaldehyde treatment. The degradable fraction (B) did not differ between regular and treated oilseed meals or between soyabean meal and rapeseed meal. The fractional rate-constant (C) of crude protein was significantly lower ($P < 0·05$) in the soyabean meals treated with F0·5 and F1·0 compared with the regular soyabean meal. These treatments significantly ($P < 0·05$) decreased C in rapeseed meal. The regular soyabean meal showed a significantly ($P < 0·05$) lower C value than the regular rapeseed meal. On the other hand, the difference between the oilseed meals disappeared after treatments F0·5 and F1·0.

The rapidly soluble fraction (A) of phytate P in soyabean meal was significantly ($P < 0·05$) decreased by these treatments. The regular rapeseed meal showed a significantly ($P < 0·05$) higher A value than the rapeseed meals treated with F0·3 and F0·5. The regular soyabean meal showed a significantly ($P < 0·05$) higher A value than the regular

Table 2. Ruminal degradation characteristics† of the crude protein and phytate phosphorus components of soyabean and rapeseed meals in their regular forms and treated with formaldehyde at levels of 3, 5 and 10 g/kg (F0·3, F0·5 and F1·0 respectively)‡

(Mean values for three sheep with their pooled standard errors)

	Crude protein			Phytate phosphorus		
	A	B	C	A	B	C
Soyabean meal						
Regular	99·5^{a}	901	43·6^{a}	185·8^{a}	814^{c}	173·7^{a}
F0·3	78·5^{ab}	922	40·1^{a}	110·1^{bc}	890^{ab}	182·8^{a}
F0·5	47·0^{c}	809	7·5^{b}	85·1^{c}	915^{a}	39·7^{b}
F1·0	65·0^{bc}	935	3·7^{b}	132·3^{b}	868^{b}	29·9^{b}
Rapeseed meal						
Regular	169·9^{a}*	782	75·1^{a}*	132·2^{a}*	868^{c}*	88·6^{a}*
F0·3	94·8^{b}	905	8·3^{b}*	97·0^{b}	903^{b}	49·1^{b}*
F0·5	38·4^{c}	835	7·4^{b}	56·6^{c}	943^{a}	25·8^{c}
F1·0	43·2^{c}	687	9·4^{b}	114·2^{ab}	886^{bc}	9·7^{c}*
SEM	3·7	51	2·2	4·3	4	2·4
Statistical significance of effect ($P<$)						
Treatment	0·0001	0·8248	0·0001	0·0001	0·0001	0·0001
Oilseed meal	0·0321	0·3029	0·7219	0·0010	0·0010	0·0001
Interaction	0·0003	0·6703	0·0002	0·2255	0·2255	0·0001

^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different, $P<0·05$.
Mean values were significantly different from those for soyabean meal treated with the same level of formaldehyde: * $P<0·05$.
† A, rapidly soluble fraction (g/kg); B, degradable fraction (g/kg); C, fractional rate-constant (g/kg per h).
‡ For details of diets and procedures, see pp. 467–468.

rapeseed meal, but the difference between the oilseed meals disappeared after treatment. The degradable fraction (B) of phytate P was significantly ($P<0·05$) lower in the regular than in the treated soyabean meal. Phytate P in the regular rapeseed meal showed a significantly ($P<0·05$) lower B value as compared with the rapeseed meals treated with F0·3 and F0·5. The regular soyabean meal showed a significantly ($P<0·05$) lower B value than the regular rapeseed meal, but the difference between the oilseed meals disappeared after treatment. The fractional rate-constant (C) of phytate P was significantly lower ($P<0·05$) in the soyabean meals treated with F0·5 and F1·0 compared with the regular soyabean meal. The treated rapeseed meal showed a significantly ($P<0·05$) lower C value than the regular rapeseed meal. The soyabean meals treated with F0·3 and F1·0 showed significantly ($P<0·05$) higher C values than the rapeseed meal treated with the corresponding levels of formaldehyde.

At all ruminal-passage rates examined, the soyabean meals treated with F0·5 and F1·0 showed significantly ($P<0·05$) lower ED of crude protein than the regular soyabean meal (Table 3). Treatment decreased the ED of crude protein in rapeseed meal with increasing level of

Table 3. The ruminal effective degradabilities of crude protein and phytate phosphorus, and their ratio (phytate phosphorus : crude protein) in soyabean and rapeseed meals in their regular forms and treated with formaldehyde at levels of 3, 5 and 10 g/kg (F0·3, F0·5 and F1·0 respectively) at three ruminal-passage rates†

(Values are means of three sheep with their pooled standard errors)

	Crude protein			Phytate phosphorus			Ratio		
Ruminal-passage rate (/h)...	0·02	0·05	0·08	0·02	0·05	0·08	0·02	0·05	0·08
Soyabean meal									
Regular	686^{a}	513^{a}	415^{a}	909^{a}	814^{a}	741^{a}	1·33^{a}	1·59^{a}	1·79^{a}
F0·3	679^{a}	486^{a}	385^{a}	910^{a}	808^{a}	728^{a}	1·34^{a}	1·66^{a}	1·89^{a}
F0·5	253^{b}	147^{b}	113^{b}	676^{b}	488^{b}	388^{b}	2·71^{b}	3·35^{b}	3·47^{b}
F1·0	212^{b}	130^{b}	107^{b}	626^{c}	453^{b}	368^{b}	2·96^{b}	3·49^{b}	3·44^{b}
Rapeseed meal									
Regular	780^{a}*	631^{a}*	541^{a}*	839^{a}*	686^{a}*	587^{a}*	1·07^{a}	1·09^{a}	1·09^{a}*
F0·3	360^{b}*	223^{b}*	180^{b}*	739^{b}*	545^{b}*	441^{b}*	2·06^{b}*	2·44^{b}*	2·45^{b}*
F0·5	242^{c}	135^{c}	102^{c}	585^{c}*	376^{c}*	286^{c}*	2·45^{bc}	2·83^{b}	2·85^{b}*
F1·0	138^{d}*	88^{d}*	73^{c}	401^{d}*	257^{d}*	209^{d}*	2·99^{c}	2·94^{b}	2·89^{b}*
SEM	6	5	5	6	5	5	0·08	0·08	0·07
Statistical significance of effect ($P<$)									
Treatment	0·0001	0·0001	0·0001	0·0001	0·0001	0·0001	0·0001	0·0001	0·0001
Oilseed meal	0·0001	0·0001	0·0014	0·0001	0·0001	0·0001	0·6970	0·1433	0·0146
Interaction	0·0001	0·0001	0·0001	0·0001	0·0001	0·0001	0·0757	0·0051	0·0056

^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different, $P<0·05$.
Mean values were significantly different from those for soyabean meal treated with the same level of formaldehyde: * $P<0·05$.
† For details of diets and procedures, see pp. 467–468.

formaldehyde. Although the regular rapeseed meal showed significantly ($P < 0{\cdot}05$) higher ED of crude protein than the regular soyabean meal, the rapeseed meals treated with F0·3 and F1·0 showed significantly ($P < 0{\cdot}05$) lower ED of crude protein than the soyabean meal treated with the corresponding levels of formaldehyde.

Treatments F0·5 and F1·0 significantly ($P < 0{\cdot}05$) suppressed the ED of phytate P in soyabean meal although treatment F0·3 did not affect the ED of phytate P at any ruminal-passage rate examined. Treatment decreased the ED of phytate P in rapeseed meal with increasing level of formaldehyde. Compared with the regular soyabean meal, the regular rapeseed meal showed significantly ($P < 0{\cdot}05$) lower ED of phytate P at all ruminal-passage rates. The treated rapeseed meals showed significantly ($P < 0{\cdot}05$) lower ED of phytate P than the soyabean meal treated with corresponding levels of formaldehyde.

The soyabean meal treated with F0·3 showed a similar ratio phytate P : crude protein in the ED (PCR) to the regular soyabean meal. The soyabean meals treated with F0·5 and F1·0 showed significantly ($P < 0{\cdot}05$) higher PCR values than the regular soyabean meal. Treatment of rapeseed meal significantly ($P < 0{\cdot}05$) increased PCR. The soyabean meal treated with F0·3 showed a significantly ($P < 0{\cdot}05$) lower PCR than the rapeseed meal treated with the same level of formaldehyde at all ruminal-passage rates. On the other hand, this ratio was significantly ($P < 0{\cdot}05$) lower in the rapeseed meals treated with F0·5 and F1·0 than in the soyabean meal treated with the same level of formaldehyde at k 0·08.

Discussion

Formaldehyde treatment decreased the ED of crude protein in soyabean meal and rapeseed meal at all ruminal-passage rates examined. These results are in agreement with those of previous reports indicating that treatment with formaldehyde reduced ruminal degradability of crude protein in soyabean meal (Nishimuta *et al*. 1974) and rapeseed meal (Coombe, 1985).

Formaldehyde treatment reduced the ED of phytate P in both oilseed meals at all ruminal-passage rates examined, suggesting that formaldehyde treatment suppressed the degradation of phytate in the rumen. Some researchers (Raun *et al*. 1956; Nelson *et al*. 1976) reported that ruminants readily utilized phytate P as a P source because ruminal microbes efficiently degraded phytate P to inorganic P. On the other hand, phytate P is known to be poorly utilizable in single-stomached animals (Reddy *et al*. 1982). Therefore, we considered that the phytate P escaping from ruminal degradation was not efficiently available in ruminants. Phytate was reported to form complexes with protein in oilseed meal (Thomas *et al*. 1946). The increase in ruminally undegradable protein resulted from a reduction of protein solubility in the rumen (Nishimuta *et al*. 1974). In the present study, the rapidly soluble fractions of crude protein and phytate P were decreased by formaldehyde treatment. Furthermore, the fractional rate-constants of crude protein and phytate P were also decreased by treatment. These results suggest that treatment with formaldehyde probably reduced the solubility of phytate complexes with protein. In addition, formaldehyde treatment delayed the degradation of phytate complexes during ruminal fermentation. These changes probably suppressed the ED of phytate P.

Rapeseed meal contained twice as much phytate P as soyabean meal. Furthermore, the regular rapeseed meal showed a lower ED of phytate P than regular soyabean meal. When ruminants are fed on regular rapeseed meal, a considerable amount of phytate may pass through the rumen without degradation.

Treatments F0·5 and F1·0 increased PCR of both oilseed meals. These treatments probably suppressed the degradation of protein more effectively than that of phytate. Rapeseed meal showed a lower ED of phytate P than soyabean meal with or without treatment. The regular soyabean meal showed higher PCR than the regular rapeseed meal at all ruminal-passage rates examined. Furthermore, the soyabean meals treated with F0·5 and F1·0 showed higher PCR than similarly treated rapeseed meal at the highest ruminal-passage rate. Phytate has been reported to be distributed uniformly in the protein body of soyabeans (Tombs, 1967) whilst in rapeseed a large amount of phytate is located in globoid crystals (Yiu *et al*. 1983). This different localization of phytate may be responsible for the higher ED of phytate P and PCR in soyabean meal than in rapeseed meal.

The results of the present study suggested that 743 g/kg phytate P in the rapeseed meal treated with F1·0 is likely to escape ruminal degradation and flow into the small intestine at a ruminal-passage rate of 0·05. According to a previous report indicating that the absorption coefficient of phytate P in rapeseed meal was as low as 19 % in swine (Weremko *et al*. 1997), 602 g/kg of phytate P in the treated rapeseed meal is unlikely to be absorbed in ruminants. Phytate P concentration was approximately 10 g/kg in rapeseed meal. When lactating cows are fed on a diet consisting of 100 g treated (F1·0) rapeseed meal/kg, the diet contains 1 g phytate P/kg originating from the treated rapeseed meal. Based on these data, 0·6 g phytate P/kg in this diet is likely to be excreted in faeces. As the requirement of P in lactating cows has been reported to be 0·22–0·42 % (National Research Council, 1988), a diet containing 100 g treated rapeseed meal/kg must be supplemented with 273–143 g more inorganic P/kg than a diet consisting of 100 g regular rapeseed meal/kg.

P deficiency is rare in high performance ruminants because feed is usually supplemented with a large amount of inorganic P. However, P in animal waste is becoming a concern from the aspect of environmental pollution. The reduction of dietary P to the required level is the easiest way to decrease P excretion. In these conditions, phytate P passing through the rumen must be considered.

Acknowledgements

This work was partly supported by a Grant-in-Aid (no. 09556063) from the Ministry of Education, Science, Sports and Culture of Japan.

References

Association of Official Analytical Chemists (1990) *Official Methods of Analysis*, 15th ed. Arlington, VA: AOAC.

Coombe JB (1985) Rape and sunflower seed meals as supplements for sheep fed on oat straw. *Australian Journal of Agricultural Research* **36**, 717–728.
Cromewell GL & Coffey RD (1991) Phosphorus - a key essential nutrient, yet a possible major pollutant - its central role in animal nutrition. In *Biotechnology in the Feed Industry*. *Proceedings of Alltech 7th Annual Symposium*, pp. 133–145 [TP Lyons, editor]. Nicholasville, KY: Alltech Technical Publications.
Ellis LC & Tillman AD (1961) Utilization of phytin phosphorus in wheat bran by sheep. *Journal of Animal Science* **20**, 606–607.
McDonald I (1981) A revised model for the estimation of protein degradability in the rumen. *Journal of Agricultural Science, Cambridge* **96,** 251–252.
National Research Council (1988) *Nutrient Requirements of Dairy Cattle*, 6th revised ed., pp. 81–88. Washington, DC: National Academy Press.
Nelson TS, Daniels LB, Hall JR & Shields LG (1976) Hydrolysis of natural phytate phosphorus in the digestive tract of calves. *Journal of Animal Science* **42**, 1509–1512.
Nishimuta JF, Ely DG & Boling JA (1974) Ruminal bypass of dietary soybean protein treated with heat, formalin and tannic acid. *Journal of Animal Science* **39**, 952–957.
Ørskov ER & McDonald I (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science, Cambridge* **92**, 499–503.
Raun A, Cheng E & Burroughs W (1956) Phytate phosphorus hydrolysis and availability to rumen microorganisms. *Agriculture and Food Chemistry* **4**, 869–871.
Reddy NR, Sathe SK & Salunkhe DK (1982) Phytate in legumes and cereals. In *Advances in Food Research*, vol. 28, pp. 1–92 [CO Chichester, EM Mark and GF Stewart, editors]. New York, NY and London: Academic Press.
Simons PCM, Versteegh HAJ, Jongbloed AW, Kemme PA, Slump P, Bos KD, Wolters MGE, Beudeker RF & Verschoor GJ (1990) Improvement of phosphorus availability by microbial phytase in broilers and pigs. *British Journal of Nutrition* **64**, 525–540.
Spears JW, Hatfield EE & Clark JH (1980) Influence of formaldehyde treatment of soybean meal on performance of growing steers and protein availability in the chick. *Journal of Animal Science* **50**, 750–755.
Thomas DF, Walter AP & George WI (1946) Protein–phytic acid relationship in peanuts and cottonseed. *Journal of Biological Chemistry* **164**, 487–507.
Tombs MP (1967) Protein bodies of the soybean. *Plant Physiology* **42**, 797–813.
Weremko D, Fandrejewski H, Zebrowska T, Han InK, Kim JH & Cho WT (1997) Bioavailability of phosphorus in feeds of plant origin for pigs - a review. *Asian-Australasian Journal of Animal Science* **10**, 551–566.
Yiu SH, Altosaar I & Fulcher RG (1983) The effects of commercial processing on the structure and microchemical organization of rapeseed. *Food Microstructure* **2**, 165–173.