Ester- and ether-linked phenolic acids in orchardgrass (*Dactylis glomerata* L.) and their digestion from cell walls when fed to sheep

Tsuneo Kondo, Tomoko Ohshita, and Tadashi Kyuma, 1991. **Ester- and ether-linked phenolic acids in orchardgrass (*Dactylis glomerata* L.) and their digestion from cell walls when fed to sheep.** Can. J. Plant Sci. 71: 1179–1182. Ester- and ether-linked *p*-coumaric (PCA) and ferulic acids (FA) in cell wall materials from orchardgrass (*Dactylis glomerata* L.), hays harvested at the heading stage and feces samples from sheep fed the orchardgrass hays were analyzed. Results showed that ether-linked PCA and FA were more resistant to ruminant digestion and enzymatic degradation than ester-linked PCA and FA.

Key words: *p*-coumaric acid, esters, ethers, ferulic acid, orchardgrass, sheep digestion

Tsuneo Kondo, Tomoko Ohshita, et Tadashi Kyuma, 1991. **Digestion des esters et des éthers d'acides phénoliques contenus dans les parois cellulaires du dactyle pelotonné (*Dactylis glomerata* L.) chez le mouton.** Can. J. Plant Sci. 71: 1179–1182. On a analysé les esters et les éthers de l'acide *p*-coumarique (APC), et de l'acide férulique (AF) dans les matériaux membranaires du foin de dactyle pelotonné (*Dactylis glomerata* L.) récolté au stade de l'épiaison, ainsi que dans les fèces des moutons nourris de ce foin. Les résultats obtenus montrent que la forme éther de l'APC et de l'AF est plus résistante à la digestion ruminale et à la dégradation enzymatique que la forme ester de ces deux acides.

Mots clés: acide *p*-coumarique, esters, éthers, acide férulique, dactyle pelotonné, digestion par le mouton

Grass cell walls are characterized by high levels of ester-linked *p*-coumaric (PCA) and ferulic acids (FA) (Higuchi et al. 1967; Hartley 1972), which are considered to be bound primarily to lignin and hemicellulose, respectively (Kato et al. 1984). Experiments demonstrated that PCA and FA esters inhibit enzymatic and microbial degradation of cell wall polysaccharides (Sawai et al. 1983; Jung and Sahlu 1986). The two esters were found to be solubilized and disappear during ruminant digestion to different extents (Jung et al. 1983; Bourquin et al. 1990).

It has been reported that grass cell walls also contain significant amounts of ether-linked PCA and FA (Scalbert et al. 1985; Kondo et al. 1990a,b). These ethers possibly act as a bridge unit between lignin and hemicellulose (Scalbert et al. 1985). No information is provided on their effects on rumen fermentation. The objective of the

present study was to examine the digestion of ester- and ether-linked PCA and FA in forage grass cell walls in ruminants and the release of these cell wall-bound phenolic acids by enzymatic treatment.

Orchardgrass (*Dactylis glomerata* L. 'Okamidori') was grown under field conditions as a pure sward at the Tohoku National Agricultural Experiment Station, located in the northern part of Japan. Orchardgrass was harvested at the heading stage in the first growth and artificially dried in 1989 and 1990. In vivo digestibility of each of the orchardgrass hays was determined by the total collection method using three different sheep (*Ovis aries*). Representative samples of the hays and feces for each sheep were oven-dried at 55°C and ground to pass a 1-mm screen. Cell wall materials were prepared as neutral detergent fiber (NDF) from the ground samples by the method of Goering and Van Soest (1970) except that decahydronaphthalene and sodium sulfite were omitted. The mean dry matter and NDF digestibilities and NDF

concentration of the two orchardgrass hays from different harvest years were 58.9%, 58.1% and 648 g kg⁻¹ dry matter, respectively.

For the hay samples, duplicate NDF preparations were digested with a commercial cellulase preparation (0.2% wt vol⁻¹ Cellulase Onozuka R-10, Yakult Honsha Co., Ltd, partially purified by gel-filtration with Sephadex G-25) in 0.05 M acetate buffer (pH 4.6) at 30°C for 16 h. The supernatant solution obtained by centrifugation was passed through Dowex 50W-X8 (H⁺ form) to remove enzyme proteins and sodium ions, and then freeze-dried to give enzyme-soluble NDF. The mean yield of the enzyme-soluble NDF of the two hay samples from different harvest years was 291 g kg⁻¹ NDF.

Ester-linked PCA and FA were extracted from the NDF samples with 1 N sodium hydroxide in the dark under N₂ at 20°C for 16 h with occasional shaking. After centrifugation, the supernatant was acidified with 6 N hydrochloric acid and phenolic acids in the acidified solution were re-covered with ethyl ether.

Ether-linked PCA and FA were analyzed in saponified NDF samples prepared according to the following procedure. The NDF sample was treated with 1 N sodium hydroxide in the dark under N₂ at 20°C for 16 h with occasional shaking. After centrifugation and filtration, the residue was washed with water and acetone, and then dried to give an alkali-insoluble fraction. The filtrate and water-washings were combined, passed through Dowex 50W-X8 (H⁺ form) to remove sodium ions and acidified to pH 2.5

with acetic acid. The acidified solution was extracted with ethyl ether, and the aqueous phase was then freeze-dried to give an alkali-soluble fraction. The alkali-soluble and -insoluble fractions were hydrolyzed with dioxane-2 N hydrochloric acid (9:1 vol vol⁻¹) for 1 h as described previously (Kondo et al. 1990b).

PCA and FA released by hydrolytic treatment were analyzed by high performance liquid chromatography. A reverse-phase column (Inertsil ODS-2, 250 × 4.6 mm i.d., Gasukuro Kogyo, Inc.) was used to separate phenolic acids with 25% aqueous acetonitrile acidified to pH 2.5 with trifluoroacetic acid as eluent. The column was operated at 40°C at a flow rate of 1.0 mL min⁻¹. Phenolic acids were monitored at 320 nm.

Table 1 shows the amounts of PCA and FA in the orchardgrass cell walls and their apparent digestion from cell walls when fed to sheep. The total amount of the detected PCA was 4 g kg⁻¹ NDF. Ether-linked PCA accounted for 10% of the total amount of the detected PCA. FA was liberated in a total yield of 7 g kg⁻¹ NDF. 21% of the detected FA was represented by ether-linked FA. Previously, we reported that, in the cell walls of Italian ryegrass stems, ether-linked FA accounted for 19-48% of the detected FA (5.4-6.5 g kg⁻¹ cell walls), whereas ether-linked PCA accounted for less than 10% of the detected PCA (4.8-7.3 g kg⁻¹ cell walls) (Kondo et al. 1990b).

The extent of the digestion of ester-linked FA from cell wall was greater than that of ester-linked PCA (Table 1). Similar results

Table 1. Amounts of ester- and ether-linked *p*-coumaric and ferulic acids in orchardgrass cell walls and their apparent digestibilities in sheep

Phenolic acid		Amount (g kg ⁻¹ NDF)	Apparent digestibility (%)
Ester-linked	<i>p</i> -Coumaric acid	3.57 ± 0.18	45.8 ± 0.5
	Ferulic acid	5.50 ± 0.40	77.4 ± 1.6
Ether-linked	<i>p</i> -Coumaric acid	0.39 ± 0.02	29.5 ± 0.0
	Ferulic acid	1.46 ± 0.11	45.3 ± 0.3
Total	<i>p</i> -Coumaric acid	3.96 ± 0.16	44.2 ± 0.6
	Ferulic acid	6.96 ± 0.52	70.8 ± 0.2

Values are expressed as means ± SD for the two orchardgrass hay samples from different harvest years.

were obtained by Jung et al. (1983) and Bourquin et al. (1990). This different digestion of PCA and FA esters is in good agreement with the finding that PCA and FA tend to be esterified to lignin and hemicellulose, respectively (Kato et al. 1984), as pointed out by Bourquin et al. (1990). Our experiment also showed that ether-linked PCA was less extensively digested than ester-linked PCA; ether-linked FA was less extensively digested than ester-linked FA.

Jung et al. (1983) observed that rapid solubilization of phenolics occurred in the rumen after feeding. When subjected to treatment with a fungal enzyme preparation having cellulase and hemicellulase activities, the orchardgrass cell walls released soluble fragments which contained more FA esters, less PCA esters and much less PCA and FA ethers than the total cell walls. The results presented in Table 2 were indicative of the higher resistance of PCA and FA ethers to the enzymatic treatment.

The present study indicates that the release of PCA and FA by the enzymatic degradation of orchardgrass cell walls was consistent with the digestion of these phenolic acids from the cell walls when fed to sheep. Ether-linked PCA and FA were more resistant to ruminant digestion and enzymatic degradation than ester-linked PCA and FA. According to Scalbert et al. (1985), FA etherified to lignin possibly forms cross-links between lignin and hemicellulose by esterification of their carboxyl group to hemicellulose in grass cell walls. Cross-links involving ether and ester linkages were suggested to restrict enzymatic

degradation of forage grass hemicellulose (Kondo et al. 1990a). On the basis of these findings, the higher resistance of PCA and FA ethers found in our study seems to be closely associated with their bonding forms and sites in the cell walls.

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Table 2. Amounts of ester- and ether-linked *p*-coumaric and ferulic acids in enzyme-soluble cell walls and their proportions relative to the amounts in total cell walls

Phenolic acid		Amount (g kg ⁻¹ enzyme-soluble NDF)	Proportion (%)
Ester-linked	<i>p</i> -Coumaric acid	2.10±0.31	17.1±1.7
	Ferulic acid	6.72±0.63	35.5±0.6
Ether-linked	<i>p</i> -Coumaric acid	0.06±0.00	4.6±0.1
	Ferulic acid	0.39±0.01	7.8±0.4
Total	<i>p</i> -Coumaric acid	2.16±0.31	15.9±1.6
	Ferulic acid	7.11±0.64	29.7±0.3

Values are expressed as means±SD for the two orchardgrass hay samples from different harvest years.

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