RELATIONSHIPS BETWEEN RUMEN DEGRADABILITY AND CELL WALL-BINDING FERULIC ACID ETHERS IN VARIOUS FORAGE CROPS

M.Goto
Faculty of Bioresources, Mie University, Tsu City 514, Japan

ABSTRACT
Ether-linked ferulic acid was determined in whole straw, plant parts and specific tissue cell walls of five barley (*Hordium vulgare* L.) cultivars and in sorghum stalks (*Sorghum bicolor* Moench x *Sorghum sudanense* Stapf) with five different growth and maturation stages and was related to their dry matter degradability (DMD) and extent of alkali-labile substitution at 0-5 arabinose residues previously determined.

KEYWORDS
Dioxane-2M HCl hydrolysis, dry matter degradability, ferulic acid ethers, lignin-carbohydrate complex, maize, sorghum

INTRODUCTION
A simple method to estimate cell wall-binding ferulic acid ethers appears to provide the routine method capable of identifying the ferulic acid bridges in forage lignin-carbohydrate complex and differences in the cell wall degradability of Gramineae (Goto et al.,1994). The DMD of barley straws (Goto et al.,1991a) and sorghum stalks (Goto et al.,1991b) was previously found to inversely respond to the extent of alkali-labile substitution at 0-5 arabinose residues, thought to be carbohydrate sites in forage lignin-carbohydrate complex (Chesson et al.,1985). It has been also shown on the barley straw that the more degradable cultivar had higher DM composition of leaf blade and leaf sheath than the less degradable cultivar, and the higher degradability of stem fraction was closely related to its higher cell density, and cell wall thickness of parenchyma tissues. It is therefore of interest to determine whether such ether-linked ferulic acid of the barley and sorghum plants is related to their DMD and extent of alkali-labile substitution at 0-5 arabinose residues. Whether the heterogeneity in the cell wall structure of specific tissue walls of barley straw extends to differences in the extent to which ferulic acid is ether linked to cell walls was also examined.

MATERIALS AND METHODS
The samples of five spring barley cultivars including whole straw, three plant parts, two types of specific tissue cell walls of the stem and of sorghum stalks harvested at five growth and maturation stages were used in this investigation. To determine ether-linked ferulic acid, the samples were separately mixed with 1M NaOH and incubated under N_2 at room temperature overnight. The alkali-insoluble residue was neutralized, washed with water and freeze-dried. The alkali-soluble fraction was acidified to pH2 and chilled quickly to enhance precipitation. After thawing, the acid-precipitated residue was recovered by centrifugation, washed with water, and freeze-dried. The two fractions were treated with a 9:1 (v/v) mixture of dioxane and 2M HCl at 87°C for 1h (Scalbert et al., 1985), and the ferulic acid released were extracted into diethyl ether and measured by gas chromatography (Goto et al., 1994). Enzymic degradation of specific tissue cell walls was determined by incubating samples with crude enzyme preparation from Ruminococcus albus F-40.

RESULTS
The amounts of ether-linked ferulic acid and alkali-labile substitution of 0-5 arabinose residues and DMD of barley and sorghum plants are shown in Table 1. The less degradable barley cultivars had nearly twice the ferulic acid content of the more degradable cultivars. A large difference in ether-linked ferulic acid content between Golden Promise and Doublet observed in whole straw was reflected in the stem fraction, but was not so pronounced in the leaf blade and leaf sheath. The content of such ferulic acid ethers was also reflected in variations in DMD between leaf blade, leaf sheath and stem from the two barley cultivars. Ether-linked ferulic acid was closely inversely related to DMD of the five barley straw cultivars, showing a higher coefficient value compared to that of arabinose residues carrying alkali-labile substituents at position 0-5. Since the lignin carbohydrate matrix structure in plant tissues is associated with protection of the cell walls against enzymic degradation, the isolated 'parenchyma' and 'fibrous tissue' cell walls were exposed to an extracellular enzyme extract from one of the major cellulolytic bacteria in the rumen. The parenchyma cell wall fraction was degraded to a lesser extent by the enzyme extract than was the fibrous tissue wall fraction. This was unexpected, because the parenchymal cell wall fraction contained less cell wall-binding ferulic acid ethers than did the fibrous tissue wall fraction, but was probably attributable to the relatively higher composition of middle lamella in parenchyma tissue. The same relationships among DMD, ether-linked ferulic acid and alkali-labile substitution of 0-5 arabinose residues were also observed for sorghum stalks.

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Table 1
Amounts of ferulic acid ethers and alkali-labile substitution of 0-5 arabinose residues and DMD of barley straw cultivars and sorghum stalks.

	DMD(g kg $^{-1}$) [w]	Proportion of alkali-labile substituents to 0-5 arabinoses(%) [w]	Ferulic acid ethers (mmol kg $^{-1}$)
Sorghum growth stage			
4th-L [x]	845.9	39.0	3.0
7th-L	676.6	57.0	10.5
Heading	630.0	53.0	13.8
Milk-ripe	574.2	60.0	16.8
Dough	521.9	59.0	19.7
Barley cultivars			
Golden promise	370.5	48.0	23.7
Golf	466.5	44.0	14.7
Klaxon	447.3	44.0	19.5
Heriot	518.4	41.0	11.9
Doublet	544.5	44.0	11.0
Morphological fraction			
Golden promise			
Leaf blade	632.0	ND [y]	3.7
Leaf sheath	741.0	ND	4.2
Stem	283.0	ND	11.6
Doublet			
Leaf blade	711.0	ND	3.5
Leaf sheath	711.0	ND	3.7
Stem	432.0	ND	9.1
Barley stem fraction			
Parenchyma walls	20.4 [z]	ND	18.5
Fibrous walls	25.9	ND	24.4

[w] The values were determined previously (Goto et al., 1991a; 1991b).
[x] 4th-L denotes the 4th leaf elongation stage etc. [y] ND; not determined
[z] Enzymic degradation was estimated by released reduce sugars μg (g mg^{-1} ml^{-1}).