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EFFECT OF CONDENSED TANNIN IN LOTUS CORNICULATUS AND LOTUS PEDUNCULATUS ON DIGESTION OF RUBISCO IN THE RUMEN

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ABSTRACT

The *in vitro* precipitation of ribulose-1,5-bisphosphate carboxylase (Rubisco) by condensed tannin (CT) extracted from *Lotus corniculatus* and *Lotus pedunculatus* and the effect of these CT on the *in vitro* rumen degradation of Rubisco was used to compare the reactivity of these CT. The chemical structure of CT from *Lotus corniculatus* was homogenous with epicatechin stereochemistry and mostly procyanidin units. The CT from *Lotus pedunculatus* was heterogenous with mostly prodelphinidin units. The amount of CT required to precipitate all the Rubisco when total soluble leaf protein was incubated with CT from *Lotus corniculatus* and *Lotus pedunculatus* was similar. Although CT from both species were able to reduce the *in vitro* degradation of Rubisco, CT from *Lotus corniculatus* was less effective than CT from *Lotus pedunculatus* at reducing this degradation.

KEYWORDS

Condensed tannin, protein degradation, reactivity, Rubisco, rumen

INTRODUCTION

The CT in *Lotus corniculatus* (22 g CT kg^{-1} dry matter (DM); Waghorn *et al*. 1987) fed to sheep, decreased nitrogen (N) digestibility by 8 percentage units but increased apparent absorption of essential amino acids (EAA) from the small intestine by 63%. The CT in *Lotus pedunculatus* (55 g CT kg^{-1} DM; Waghorn *et al.* 1994) also reduced N digestibility but the apparent absorption of EAA from the small intestine was unaffected by CT. In sheep fed ryegrass (Lolium perenne) and *Lotus pedunculatus* with a final dietary CT concentration of 18 g CT kg^{-1} DM, N digestibility was reduced by 13 percentage units (Waghorn and Shelton 1995) and the effects of CT were similar to *Lotus pedunculatus* (55 g CT kg^{-1} DM) fed to sheep. Nutritional responses to CT are affected by concentration and the chemical structure and reactivity of CT. The objective of this study was to measure the reactivity of CT extracted from *Lotus corniculatus* and *Lotus pedunculatus.* The in vitro precipitation of Rubisco by CT and the effect of CT on the *in vitro* degradation of Rubisco when total soluble plant protein was incubated with rumen fluid was used to compare the reactivity of CT. Rubisco is the principal leaf protein, representing 30-50% of the total protein present (Mangan, 1982).

METHODS

Condensed tannins were extracted from *Lotus corniculatus* and *Lotus pedunculatus* and total soluble leaf protein was extracted from white clover (*Trifolium repens*) using the methods described by Jackson *et al.* (1996). The chemical structure of the CT were analysed by ^{13}C-Nuclear Magnetic Resonance (NMR) and by thin layer chromatography (TLC) according to the methods described by Foo *et al.* (1996).

The precipitation of Rubisco by CT was determined by adding either 0, 0.1, 0.5, 1, 10, 25 or 50 µg of the CT extracted from *Lotus corniculatus* and *Lotus pedunculatus* to 10 mg of total soluble leaf protein. Barry and Forss (1983) reported that 1.8-2 mg of polyethylene glycol (PEG) g^{-1} CT was sufficient to release all protein from CT-protein complexes. Therefore, identical reactions were also prepared except 100 µg of PEG (molecular weight (MW) 3500) was added to remove all the effects of the CT. All reactions were diluted to 100 ml with McIlvaineís Buffer, pH 7.0 (Elving *et al.* 1956) and incubated at 39°C for 90 min. After incubation reactions were centrifuged at 12,000 g for 10 min and the resulting pellets were washed three times in McIlvaineís Buffer, pH 7.0 to remove all soluble leaf protein that was not precipitated by CT.

The effect of CT on the degradation of Rubisco was measured using *in vitro* rumen incubations. A sheep with a large rumen cannula and fed fresh perennial ryegrass/white clover pasture *ad. lib*. was used to supply rumen fluid for the incubations which were carried out using a modification of the method described by McNabb *et al.* (1996). Either 0, 0.5, 1, 5 and 10 mg of CT from *Lotus corniculatus* and *Lotus pedunculatus* were incubated with 5.6 mg of total soluble leaf protein and 0.75 ml of rumen fluid. The incubations were diluted to 3.75 ml with artificial saliva (McDougall, 1948) and incubated at 39°C for 8 h. Identical incubations were undertaken but with 20 µg of PEG added.

Both *in vitro* experiments were undertaken in duplicate. Pellets and *in vitro* rumen samples were resuspended in loading buffer (McNabb *et al.* 1996) and stored at -20oC for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein was fractionated by SDS-PAGE and the Rubisco quantified by imaging densitometry (BioRad, Model GS-670 Imaging Densitometer with Molecular Analyst$_{TM/CP}$ imaging analysis software) using methods described by McNabb *et al.* (1996).

RESULTS AND DISCUSSION

The distinguishing features of the chemical structure of the CT from *Lotus corniculatus* were its relatively homogenous monomer units with epicatechin type stereochemistry and the predominance of procyanidin units. In contrast, CT from *Lotus pedunculatus* was more heterogenous with the monomer units being a mixture of epicatechin and catechin type stereochemistry and a clear dominance of prodelphinidin to procyanidin units. The reactivity of CT increases with increasing delphinidin: cyanidin (PD:PC) ratio (Jones *et al.* 1976). Therefore, the CT from Lotus pedunculatus should be able to precipitate more protein per unit weight than the CT from *Lotus corniculatus*.

The *in vitro* precipitation of Rubisco by CT from *Lotus corniculatus* and *Lotus pedunculatus* is shown in Fig. 1, whilst the effect of these CT on the *in vitro* degradation of Rubisco when total plant protein was incubated with rumen fluid is shown in Fig. 2. The amount of CT required to precipitate all the Rubisco when 10 µg of total soluble leaf protein was incubated with CT from *Lotus corniculatus* and *Lotus pedunculatus* was similar, with between 25 and 50 µg required. Reactivity, which can be defined as the ability of CT to precipitate protein per unit weight, was similar for the CT from *Lotus corniculatus* and *Lotus pedunculatus*.

However, there were considerable differences in the effect of CT from *Lotus corniculatus* and *Lotus pedunculatus* on the *in vitro* degradation of Rubisco by microorganisms in rumen fluid. The inclusion of 5 or 10 mg of *Lotus corniculatus* CT reduced the degradation of Rubisco to 54% and 61% of the protein added to the incubation, respectively, whilst Lotus pedunculatus CT (5 mg and 10 mg) reduced the degradation of Rubisco to 28% of the protein added to the incubation. When 5 mg and 10 mg of CT was added to the *in vitro* rumen degradation assay, the ratio of CT to protein was about 1:1 and 2:1, respectively. At these ratios, the precipitation of Rubisco by CT from *Lotus corniculatus* and *Lotus pedunculatus* was similar, and about 0.9. However, CT from *Lotus corniculatus* was less effective than CT from *Lotus pedunculatus* at reducing the

degradation of Rubisco by rumen microorganisms. This suggests that factors other than just protein precipitation may be important in determining the final effect of CT in the rumen of sheep.

The precipitation of Rubisco by CT from *Lotus corniculatus* was completely reversible by PEG. However, about 0.2 of the Rubisco was still precipitated when 50 µg of CT from *Lotus pedunculatus* was incubated with PEG. Jackson *et al.* (1996) demonstrated that CT prepared from a range of forages by affinity chromatography using Sephadex LH-20 as a matrix, contained variable amounts of non-CT phenolic compounds together with the CT. Similar compounds may have been present in the CT which was extracted and purified from *Lotus pedunculatus* and may have been responsible for precipitating Rubisco in the presence of PEG.

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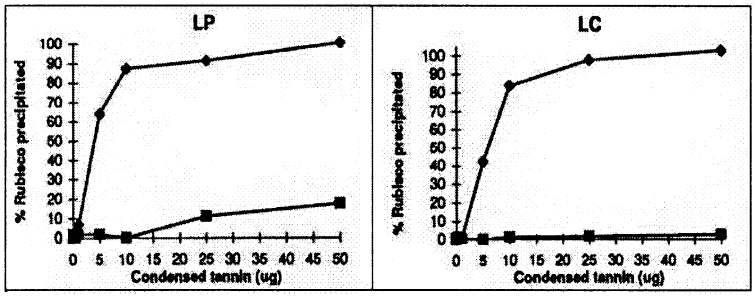

Figure 1
The proportion of ribulose-1,5-bisphosphate carboxylase (Rubisco) protein precipitated when condensed tannins (CT) extracted from *Lotus corniculatus* **(LC)** and *Lotus pedunculatus* **(LP)** were incubated with total soluble leaf protein extracted from white clover. The incubations were done with (- - -) and without (- - -) the addition of polyethylene glycol (PEG; molecular weight (MW) 3500). All incubations were done in duplicate.

Figure 2
The proportion of ribulose-1,5-bisphosphate carboxylase (Rubisco) protein degraded by rumen microorganisms when condensed tannins (CT) extracted from *Lotus corniculatus* **(LC)** and *Lotus pedunculatus* **(LP)** were incubated with total soluble leaf protein extracted from white clover (*Trifolium repens*) and rumen fluid from a sheep fed fresh perennial ryegrass (*Lolium perenne*)/white clover pasture. The incubations were done with ■(- ■ - -) and without (- - -) the addition of polyethylene glycol (PEG; molecular weight (MW) 3500). All incubations were done in duplicate.