Animal Feed Science and Technology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

A new index to estimate reactivity and biological effect of tannins, using tropical browse legumes as a model

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ARTICLE INFO

Article history:
Received 9 December 2014
Received in revised form 8 April 2015
Accepted 9 April 2015
Available online xxx

Keywords:
Tannins
Reactivity
Tropical legumes
Fermentation
Modelling

ABSTRACT

The aim of this paper was to propose a new approach to evaluate the effect of tannins on rumen microbial fermentation in terms of biological effect (proportion of the response if tannins are inactivated, *BE*) and reactivity (capability of tannins to bind chemically with different amounts of a reagent), by recording gas production in 24 h *in vitro* incubation of substrates with increasing levels (0.025–1 g/g substrate) of polyethylene glycol (PEG). Three tropical browse legumes with different tannin contents (*Acacia cornigera, Albizia lebbekoides* and *Leucaena leucocephala*) were chosen as model substrates. The reactivity index (*RI*, mg PEG) and absolute reactivity index (*aRI*, mg PEG/ml gas) were estimated from the rate coefficient of fitting gas production per unit of added PEG to an exponential equation for each incubation time. The *RI* characterises each specific tannin source, and depends on the potential fermentation of the plant. The average *RI* values of *A. cornigera*, *L. leucocephala* and *A. lebbekoides* were 70.4, 36.3 and 55.7 mg PEG, respectively. The *aRI* is a tool for species comparison, because it is referred to the total volume of gas produced. It was higher in *A. lebbekoides*, intermediate in *A. cornigera* and lowest in *L. leucocephala* (1.57, 0.76 and 1.14 mg PEG/ml gas, respectively; P=0.012). This index was higher at 6 h than at 12 and 24 h (1.62, 0.96 and 0.89 mg PEG/ml gas, P<0.001). The estimation of these indexes is a simple and inexpensive tool that gives a more complete view of the biological activity pattern of tannins in practical conditions, complementing that of other biological effect indexes and avoiding the biases that arise from their chemical analysis.

1. Introduction

The main limitation of browse legumes for being used as feed for ruminants is their content in antinutritional compounds such as tannins, which can reduce feed intake and nutrient digestibility (Stürm et al., 2007; Tiemann et al., 2008). Because of the very heterogeneous chemical structure of these phenolic compounds (McSweeney et al., 2001; Mueller-Harvey, 2006), tannins of different origin may differently affect nutrient availability and utilisation, even when consumed at the same concentration (Schofield et al., 2001).

Abbreviations: *aRI*, absolute reactivity index; *eBE*, estimated biological effect; *gBE*, biological effect calculated from gas production; DM, dry matter; OM, organic matter; PEG, polyethylene glycol; *RI*, reactivity index.

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http://dx.doi.org/10.1016/j.anifeedsci.2015.04.007
0377-8401/

Since the reactivity of tannins is determined by their chemical structure (Kraus et al., 2003), several chemical analyses have been attempted to estimate their activity; however, most rapid colorimetric methods provide scarce information on this issue (Álvarez del Pino et al., 2005; Mueller-Harvey, 2006). Recent approaches (*e.g.*, Gea et al., 2011) combine high performance liquid chromatography (HPLC) with thiolytic degradation to obtain quantitative and qualitative information in terms of degree of polymerisation and procyanidin/prodelphinidin- and cis/trans-ratios, but such procedures are very laborious and expensive. Simpler and inexpensive methods are necessary to estimate tannin reactivity and nutritive value of browse legumes for ruminant feeding.

The *in vitro* gas production technique combined with a tannin binding agent (polyethylene glycol; PEG) has been used as a bioassay to provide an index of the biological effect of tannins on microbial utilisation of feeds (Makkar et al., 1995; Rodríguez et al., 2014). Nevertheless, though relatively easy and inexpensive, this method based only on gas production measurements may give limited information on tannin biological impacts (Makkar, 2005), as it does not discriminate if the effect of tannin depends merely on its concentration or it is also affected by its specific physical–chemical nature. Since differences in reactivity of tannins of different nature, even present at the same amount, would be apparent along the fermentation process, we hypothesised that the *in vitro* fractional rate of gas production (c) would be better related to tannin reactivity than the gas production itself. Thus, this work was conducted to test the suitability of an index based on the response of c to the gradual inactivation of tannins by the addition of increasing levels of PEG to predict the biological activity of these phenolic compounds in three tropical browse legumes, chosen as a model.

2. Material and methods

Leaves and small twigs (<5 cm) from the browse legumes *Acacia cornigera*, *Albizia lebbekoides* and *Leucaena leucocephala*, previously assayed by Rodríguez et al. (2010, 2011, 2014), were studied. Samples (up to 200 g) were collected from four trees from each species, grown at the Institute of Animal Science (ICA, San Jose de las Lajas, Cuba), pooled, dried at 60 °C for 48 h and ground to pass a 1 mm screen. Their total phenolic and tannin content was analysed following the colorimetric method of Makkar et al. (1993) using the Folin-Ciocalteau reagent and with tannic acid (MERCK Chemicals, Madrid, Spain) as the reference standard.

Substrates (800 mg) were incubated anaerobically following Theodorou et al. (1994), in bottles with 80 ml of an incubation solution without microminerals and resazurin solutions, and with HCl-cysteine as the reducing agent (Mould et al., 2005). The rumen inoculum was included at 0.20 of the total incubation volume. Legumes were incubated with up to 10 levels (0, 20, 40, 80, 120, 160, 240, 360, 480 and 800 mg) of PEG 6000 (PANREAC, Barcelona, Spain), corresponding to proportions of 0, 0.025, 0.05, 0.10, 0.15, 0.20, 0.30, 0.45, 0.60 and 1.00 of the incubated vegetal substrate. Five incubation series of 24 h were carried out, with at least six levels of PEG tested simultaneously, in duplicates. Internal pressure of bottles was recorded at 2, 4, 6, 8, 10, 12, 16 and 24 h with an HD2124.2 manometer equipped with a TP704 pressure gauge (DELTA OHM, Caselle di Selvazzano, Italy). Pressure readings were converted into volume by linear regression (Rodríguez et al., 2011).

2.1.1. Calculations and statistical analysis

The reactivity of tannins from each of the three legume species at a given time was estimated by relating gas production with the level of PEG included, following the model:

$$Y = a + b(1 - \exp(-cx)), \tag{1}$$

where Y is the accumulated gas production (mL/g organic matter, OM) at an x level of PEG inclusion; a is the gas production from the incubated substrate in absence of PEG (without tannin inactivation); b is the increase of gas production from the substrate when tannins are completely inactivated (maximum dose of PEG); and c is the fractional rate of gas production according to PEG addition. The standard errors (SE), and variation (cv) and correlation (R) coefficients of the equations for each fitted curve were also estimated.

Assuming that the reactivity of tannins from a given vegetal species and their inactivation by PEG are inversely related (Muetzel and Becker, 2004) and that 1 g PEG/g substrate completely inactivates tannins (Makkar et al., 1995), reactivity was defined by the equation:

$$R = \lambda \left(\frac{1}{c}\right), \tag{2}$$

where the reactivity of tannins from a given species (R) is determined by changes in gas production when the activity of tannins against fermentation is inactivated by increasing levels of added PEG, and λ is a proportionality constant specific for each vegetal species. The coefficient c corresponds to the fractional rate from Eq. (1). Since both R and λ are constant and specific for each plant substrate, it was assumed that their ratio is the reactivity index (RI) for each species, which can be estimated from Eq. (2) as:

$$RI = \frac{R}{\lambda} = \left(\frac{1}{c}\right), \tag{3}$$

Please cite this article in press as: Rodríguez, R., et al., A new index to estimate reactivity and biological effect of tannins, using tropical browse legumes as a model. Anim. Feed Sci. Tech. (2015), http://dx.doi.org/10.1016/j.anifeedsci.2015.04.007