Animal Feed Science and Technology 228 (2017) 178–185

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Complementarities between grasses and forage legumes from temperate and subtropical areas on *in vitro* rumen fermentation characteristics

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ARTICLE INFO

Keywords:
Grass-legume mixtures
Tropical forage
Temperate forage
Condensed tannins
In vitro rumen fermentation
Associative effects

ABSTRACT

Associative effects between plants included in some mixtures with tropical and temperate species, which are able to grow in a wide range of temperature, deserve to be better investigated. The aim of this work was to assess the *in vitro* rumen fermentation characteristics when a tropical (peanut, *Arachis pintoi*) or temperate legume (sainfoin, *Onobrychis viciifolia*) with condensed tannins (CT), or a temperate legume without CT (alfalfa, *Medicago sativa*), were mixed with a tropical (axonopus, *Axonopus catharinenses*) or a temperate grass (tall fescue, *Festuca arundinacea*). Different proportions of each legume were mixed with each grass (in%, on dry matter (DM) basis, 0:100, 25:75, 50:50 75:25 and 100:0), and were tested using an *in vitro* rumen fermentation assay. The crude protein content in both tropical and temperate grasses ranged from 100 to 120 g/kg DM, but the aNDF content was much higher in the tropical (617 g/kg DM) than in the temperate grass (464 g/kg DM). The ADF (+214 g/kg DM) and lignin (+46.2 g/kg DM) content was much higher in the alfalfa when compared with the average of two other legumes. Positive quadratic effects were detected on *in vitro* DM disappearance (IVDMD), total gas production, and volatile fatty acids production, when alfalfa was mixed with axonopus, but similar response was not observed when alfalfa was mixed with tall fescue. The IVDMD and total gas production linearly increased when the proportion of peanut or sainfoin increased in the mixtures containing axonopus, but did not change when these legumes were mixed with tall fescue. The ammonia production increased when the proportion of legume increased in all mixtures. Reductions on ammonia and methane (CH_4) productions were observed in mixtures containing sainfoin without the presence of polyethylene glycol (PEG) compared to fermentation done with PEG, but this response was not observed in the mixtures containing peanut. We conclude that complementarities in terms of energy and protein content can create favourable conditions leading to positive associative effects on rumen digestive parameters when legume species were mixed with axonopus, but not when they are mixed with tall fescue, probably due to different fibre content of grasses. The CT of sainfoin allow to reduce ruminal protein degradability and mitigate CH_4 emissions per kg of DM, while the CT content of peanut is not enough to have the same effects.

Abbreviations: ADF, acid detergent fibre expressed inclusive of residual ash; ADL, lignin in acid detergent; aNDF, neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; CP, crude protein; CT, condensed tannins; CH_4, methane; DM, dry matter; IVDMD, *in vitro* dry matter disappearance; NH_3-N, ammonia N; OM, organic matter; PEG, polyethylene glycol; VFA, volatile fatty acids

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http://dx.doi.org/10.1016/j.anifeedsci.2017.04.020
Received 22 November 2016; Received in revised form 6 April 2017; Accepted 9 April 2017

1. Introduction

The advantages of legume inclusion in ruminant feeding on grass-based systems have been reported by several authors (Lüscher et al., 2014; Elgersma and Søegaard, 2016; Pembleton et al., 2016). Legumes can increase animal performance with environmental benefits, in particular through the symbiotic nitrogen fixation. At the animal level, the effects can vary according to the legume proportion, but also the possible occurrence of associative effects between grasses and legume species (Hunt et al., 1985; Niderkorn and Baumont, 2009). In addition, the presence of secondary metabolites in some species, such as condensed tannins (CT) allows to impact positively enteric methane (CH_4) emissions (Patra, 2010) and protein digestion. Forming natural tannin-protein complexes decreases ruminal protein degradation and urinary excretion of nitrogen by ruminants, and may lead to increase the flux of dietary protein for absorption in the intestine (Theodoridou et al., 2010). The effects of CT on rumen digestion are depending on their concentration, type and activity (Soltan et al., 2012).

Some recent studies have evaluated the associative effects in grass-based diets combining a temperate legume with a temperate grass (Niderkorn et al., 2011, 2015; Niderkorn et al., in press) or a tropical legume with a tropical grass (Dall-Orsoletta et al., 2017), but mixtures between tropical and temperate species deserve to be better investigated. This kind of work is justified because certain temperate species, such as alfalfa (*Medicago sativa*), are able to produce in a wide range of pedoclimatic conditions (Moreira et al., 2011). Thus, it is possible to find temperate plants growing in tropical or subtropical regions, as northeastern of Brazil (Pompeu et al., 2003) and South Africa (Theron and Snyman, 2015). In the same way, tropical legumes such as peanut (*Arachis pintoi*), are able to produce in subtropical regions (Jones, 1993; Ferguson and Loch, 1999). Additionally, the average temperature of several regions of the planet have been increasing (IPCC, 2014), which may increase the occurrence of tropical pastures in subtropical and temperate regions of the world.

We hypothesize that a wide range of associative effects may occur when mixing different grasses with legumes containing CT. Thus, the aim of this work was to assess the ruminal digestive parameters when temperate or tropical legumes with contrasted CT contents (negligible, low and high) were mixed with a temperate or a tropical grass differing by their fibre content.

2. Materials and methods

The study was conducted in the facilities of the UMR1213 Herbivores Research Unit of INRA Auvergne-Rhône-Alpes in Central France. All the procedures were conducted in accordance with the European Union Directive No. 63/2010 and French Guidelines for the use of experimental animals.

2.1. Experimental design

This article provides the *in vitro* rumen fermentation patterns of pure and binary mixtures of grass and legume species from contrasted climatic areas. We used two grasses, namely axonopus (*Axonopus catharinensis*, tropical specie) and tall fescue (*Festuca arundinacea*, temperate specie) and three legumes, namely peanut (*Arachis pintoi*, CT-containing tropical specie), alfalfa (*Medicago sativa*, temperate specie without CT) and sainfoin (*Onobrychis viciifolia*, CT-containing temperate specie). Each pure plant ($n = 5$) and all the possible grass-legume combinations in three proportions (in%, on dry matter (DM) basis, 25:75, 50:50 and 75:25, $n = 18$) were tested. The effects of CT were assessed by conducting the experiment with and without polyethylene glycol (PEG) in the incubation medium, a compound that can bind and inactivate CT (Makkar et al., 1995) (total: $n = 46$). Each treatment (5 pure plants and 18 binary associations) were fermented during 24 h with and without the presence of PEG. These treatment combinations were repeated three times (runs) over a period of two weeks. The total number of observations was 23 (forages) × 2 (PEG) × 3 (runs) = 138.

2.2. Plant materials

The tropical plants species (axonopus and arachis) grew in subtropical humid climate of Southern Brazil (27°25′S, 49°38′W), on a Cambissol Alic soil with 1500 mm annual rainfall and main temperature of 17 °C. The areas with arachis and axonopus received 3.2 tons/ha of turkey litter bedding and 300 kg/ha of natural phosphate, totaling 100 kg/ha of phosphorus (P), 50 kg/ha of potassium (K) and 60 kg/ha of nitrogen (N). Before sampling, these areas were mowed to achieve uniformity. After mowing, axonopus pasture received a total of 50 kg of N/ha as ammonium nitrate and no additional fertilizer was applied into arachis area. Samples of both tropical species were taken by cutting the plants at 5-cm height from the soil after 28 days of regrowth, in February of 2014.

Samples of the three temperate species (tall fescue, sainfoin and alfalfa) were from the INRA collection of freeze-dried plant substrates, which were grew at the INRA's station of Clermont-Ferrand-Theix site (45°43′N, 03°01′E, 870 m above sea level, central France,) on a granitic brown soil. Tall fescue was sown in May 2006, the sward was fertilized with 80 kg/ha of phosphorus (P), 120 kg/ha of potassium (K) and 180 kg/ha of nitrogen (N), and forage was harvested on 25 September 2007. Sainfoin and alfalfa were sown in August 2008, and harvested on 30 April 2009 during the first growth cycle. No mineral fertilizer was applied for these two legumes. All the plants were cut at 6-cm height from the soil at vegetative stage.

2.3. In vitro batch fermentation

Rumen fluid was collected in equal proportion from three cannulated sheep (Texel, adult castrated males, 61.2 ± 9 kg on