Animal Feed Science and Technology 157 (2010) 41–54

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

In vitro ruminal degradation of ricin and its effect on microbial growth

A.S. de Oliveira[a,*], M.R.C. Oliveira[b], J.M.S. Campos[c], R.P. Lana[c], O.L.T. Machado[d], C.A. Retamal[d], E. Detmann[c], S.C. Valadares Filho[c]

[a] *Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso, Campus Sinop, Sinop, MT 78557-267, Brazil*
[b] *Departamento de Bioquímica e Biologia Molecular/BIOAGRO, Universidade Federal de Viçosa, Viçosa, MG 36570, Brazil*
[c] *Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, MG 36570, Brazil*
[d] *Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ 28013-600, Brazil*

ARTICLE INFO

Article history:
Received 12 June 2009
Received in revised form
20 December 2009
Accepted 15 January 2010

Keywords:
Castorseed
Detoxification
Electrophoresis
Toxin

ABSTRACT

Ricin is a toxic protein found in castorseed (*Ricinus communis* L.). We hypothesized that ruminal microbiota are capable of degrading ricin, and that the toxin inhibits ruminal microbial growth. Therefore, first we evaluated the *in vitro* ruminal degradation of ricin from solvent castorseed meal (SCM) by SDS-PAGE and densitometry analysis of culture medium (Experiment 1). Culture medium (three replicates) were collected after 0, 3, 6, 12, 24 and 48 h of incubation content initially 0, 61, 122 and 244 μg of ricin/mL or 122 μg of ricin/mL (without ruminal inoculum). No protein compounds were detected by SDS-PAGE in the culture medium without ricin, indicating an absence of interference from the ruminal inoculum. Ricin chains remained intact in the absence of rumen inoculum, but they were degraded at rates of 0.2725, 0.1504 and 0.0648 h^{-1} with ruminal inoculum, at initial ricin concentrations of 61, 122 and 244 μg/mL. Next, the effect of ricin denaturation on rumen microbial specific growth rate (SGR) (OD-600 nm) and the average ammonia concentration at the same time of incubation were investigated (Experiment 2). This experiment had a completely randomized design in a 3 × 3 factorial (three replicates) arrangement, with three sources of protein (trypticase-control; crude extract of soluble protein at pH 3.8 buffer of solvent castorseed meal (CEP) intact, containing 1.46 mg of ricin/mL; and denatured CEP with calcium oxide, containing 0.04 mg of ricin/mL) and three protein levels (0.42, 0.84, and 1.68 mg/mL). There was interaction (P=0.021) between protein level and protein source for SGR. A linear increase (P<0.001) of SGR was observed with increase of trypticase level, but there was a quadratic effect (P=0.023) with increase of intact CEP level, with a minimum value of SGR of −0.004 h^{-1} at a protein level of 1.45 mg/mL (210 μg of ricin/mL) of intact CEP. There was no effect (P=0.099) of denatured CEP level, but SGR increased (P<0.001) 3.2 times with denaturation of intact CEP. Ruminal microbial growth was inhibited by 50% with 89 μg of ricin/mL. Ammonia concentration was 91% lower (P<0.001) for the CEP source when compared to trypticase, but the denaturation of intact CEP had no effect (P=0.9560) on the ammonia concentration. Although ruminal microbiota was able to degrade ricin in *in vitro* conditions, the toxin inhibits ruminal microbial growth. Therefore, complete detoxification of CSM before using it to feed ruminants is recommended. The denatured CEP presents potential of use as modifier of rumen microbial fermentation.

Abbreviations: CEP, crude extract of soluble protein at pH 3.8 buffer of solvent castorseed meal; OD, optical density; SCM, solvent castorseed meal; SGR, specific growth rate.

* Corresponding author at: Avenida Alexandre Ferronato, Centro de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso, Campus Sinop, Sinop-MT, no. 1200, 78557-267, Brazil. Tel.: +55 66 3531 1663; fax: +55 66 3531 9796.
E-mail address: andresoli@uol.com.br (A.S. de Oliveira).

0377-8401/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.anifeedsci.2010.01.006

1. Introduction

The main factor limiting the use of co-products of castorseeds (*Ricinus communis* L.) in animal feed is the presence of ricin. This protein toxin inactivates eukaryotic ribosome irreversibly (Olsnes et al., 1974; Endo and Tsurugi, 1988; Audi et al., 2005). In prokaryotic cells, intact ricin or isolated chains of ricin shows no toxic effects, but active polypeptides obtained from the hydrolysis of ricin by trypsin are able to inhibit protein synthesis of *Escherichia coli* (Haas-Kohn et al., 1980).

Toxic symptoms and death caused by the ingestion of castorseeds have been described in both non-ruminant and ruminant animals (Armién et al., 1996; Tokarnia and Dobereiner, 1997; Kumar et al., 2003; Aslani et al., 2007). However, several studies have reported tolerance in ruminant animals fed diets containing between 100 and 150 g/kg of DM of non-detoxified castorseed meal (Albin et al., 1969; Santana et al., 1971; Oliveira, 2008). In revision on ricin from *R. communis* as undesirable substances in animal feed realized by Scientific Opinion of the Panel on Contaminants in the Food Chain (2008), authors concluded that if acclimatised with moderate doses, cattle seem to tolerate relatively high ricin levels for long time exposure. Because of intense microbial ruminal proteolytic activity (Wallace et al., 1997), it was hypothesized that the ricin is fully or partially hydrolyzed in the rumen.

Additionally, increases in ruminal microbial protein synthesis after semi-detoxification of castorseed meal by alkaline treatment were observed in sheep, but this effect was confounded by an increase in the intake of digestible nutrients (Oliveira, 2008). The elimination of such confounding factors can be achieved by using *in vitro* systems for microbial growth. We also hypothesize that the ricin can reduce microbial protein synthesis.

To evaluate the ruminal proteolytic activity on specific proteins, it is necessary to use techniques of protein separation, identification and quantification. Among the available techniques, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis are characterized by their relative simplicity and accuracy. These techniques have been used extensively for the evaluation of ruminal degradation of specific groups of proteins in feed systems *in vitro* or *in situ*, and even allow for the estimation of kinetic parameters of degradation (Nugent et al., 1983; Spencer et al., 1988; Romagnolo et al., 1994; Messman and Weiss, 1994; Schwingel and Bates, 1996; Tai and Bush, 1997; Chiou and Wu, 1999; Sadeghi and Shawrang, 2008).

Due to the lack of information regarding the ability of ruminal microorganisms to proteolyze ricin and of ricin's effects on the ruminal microbiota, *in vitro* conditions were evaluated by two experiments: Experiment 1—the ruminal degradation of ricin from solvent castorseed meal (SCM) by SDS-PAGE and densitometry analysis, and Experiment 2—the effect of ricin denaturation from SCM on the rumen microbial specific growth rate (SGR) and the average ammonia concentration.

2. Materials and methods

2.1. Solvent castor meal sample and care animals

Solvent castorseed meal (SCM) was purchased from Brazilian agri-industrial companies located in Salvador, Bahia (BOM-Brazil Óleo de Mamona Ltd.). According to the manufacturers the SCM is marketed exclusively as organic fertilizers and are not recommended for animal feeding. The SCM was ground to pass a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA).

The experiments were conducted at the Laboratory of Animal Nutrition of the Animal Science Department and Laboratory of Protein of Department of Biochemistry and Molecular Biology/BIOAGRO of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. Care and handling of the animal, including ruminal cannulation, were conducted as outlined in the guidelines of the University Federal of Viçosa Institutional Animal Care and Use Committee.

2.2. In vitro ruminal degradation of ricin (Experiment 1)

2.2.1. Preparation of extract protein of solvent castorseed meal containing ricin

The crude extract of soluble protein at pH 3.8 buffer of solvent castorseed meal (CEP) intact concentrated (10 mg of protein/mL) was obtained as follows: one aliquot (10 g) of SCM (intact) was added to one Falcon tube (total of 30 tubes). Forty milliliters of extraction buffer (0.5 M Tris pH 3.8, adjusted with 370 mL/L of sulfuric acid) was added to each Falcon tube, and the tubes were centrifuged (Jouan BR4i multifunction, Thermo Electron Corporation, Waltham, MA, USA) at 9739 × *g* for 20 min, at room temperature. The supernatants (855 mL) were mixed and the volume was measured. After 5 mL-aliquots of supernatant had been collected for the determination of total protein and electrophoretic analysis, the remaining liquid was immediately concentrated (10× on average) in a lyophilizer (Labconco Freeze Dry System, Kansas City, MO, USA) to obtain a final protein concentration of 10 mg/mL, then resuspended in deionized water.

2.2.2. Preparation of rumen fluid and culture medium

Ruminal fluid was obtained from one rumen fistulated Nellore steer after 14 days receiving a diet [containing 700 g/kg of corn silage and 300 g/kg of concentrate, 779.0 g/kg ground corn grain, 183.0 g/kg soybean meal, 8.0 g/kg urea + ammonia sulfate, 10 g/kg sodium chloride, 10 g/kg dicalcium phosphate, 6.6 g/kg of sodium bicarbonate and 3.4 g/kg magnesium oxide] on a DM basis. Approximately 1 L of ruminal liquid was collected adhered in digesta from four positions of rumen ventral (at random), 2 h postfeeding. After collection, the liquid was filtered on four layers of cheesecloth, packed in a hermetically