Small Ruminant Research 111 (2013) 71–75

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

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

Short communication

Effect of direct-fed microbial consortia on ruminal fermentation of maize stover in sheep

F.N. Fon*, I.V. Nsahlai

Animal and Poultry Science, School of Agricultural Science and Agribusiness, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa

ARTICLE INFO

Article history:
Received 9 September 2011
Received in revised form 15 June 2012
Accepted 20 September 2012
Available online 22 October 2012

Keywords:
Transinoculation
Faecal inoculum
Degradability
Herbivore
Cellulases

ABSTRACT

Pure enzymes, antibiotics, probiotics and specific microbial strains which are expensive have been used to manipulate the rumen microbial ecosystem and its fermentation characteristics with limited success. Direct-fed microbial consortia (faecal inoculum from; N1 (horse + wildebeest, 1:1 ratio) and N3 (wildebeest + zebra, 1:1 ratio)) effects on maize stover (MS) degradation, ruminal fermentation characteristics and cellulase enzyme profiles were investigated. In experiment 1, six fistulated male Marino sheep (44 ± 1.5 kg) receiving 1.3 kg of MS and lucerne (1:0.3 ratio), mineral block and water *ad libitum*, were randomly assigned to two groups (control and treatment) of three in each. The treatment group were dosed with 50 g (fresh faeces) or 50 ml (cultured faecal inocula) of N1 every 3.5 days through the fistula. Degradation characteristics of MS were measured by incubating 3 g in nylon bags for 96, 72, 48, 24, 12, 9, 6 and 3 h in the rumen of sheep. Rumen fluid was analyzed for ruminal fermentation characteristics (pH, short chain fatty acids (SCFAs), CO_2, CH_4) and fibre degrading enzymes (exoglucanse, endoglucanse and endoxylanase) specific activities (IU/mg). In experiment 2, feeding was the same as in experiment 1 but for the inoculum (N3). Exoglucanase and endoglucanase specific activities increased ($P < 0.05$) in N1 (1.1 and 1.8 fold) and N3 (1.3 and 1.5 fold) compared to their controls. Dry matter intake (MS) increased ($P < 0.03$) in N1 but tended to increase ($P = 0.08$) in N3. No differences were observed for the insoluble but degradable fraction of MS, rate of degradation of MS, potential degradability and effective degradability in both N1 and N3 after 96 h. Total SCFA and propionic acid tended to increase in N3. Interestingly, CH_4 and CO_2 were relatively lower in both N1 (3 and 1.9%) and N3 (9 and 1.9%) respectively. Direct-fed microbials from N1 to N3 increased cellulases activity with the potential of increasing DMI. Optimization of microbial consortia can improve livestock productions at a reduced feed cost as the method is cheaper and available to all farmers especially in the developing countries.

1. Introduction

Exogenous fibrolytic enzymes hold a lot of promise as means of increasing forage utilization, milk production, average daily weight gain and improving the productive efficiency of ruminants but are limited by their hydrolysis in the rumen environment (Peters et al., 2010). However, some studies demonstrated little or no significant changes in fermentation parameters measured when supplemented with enzymes (Peters et al., 2010; Pinos-Rodriguez et al., 2008). As the use of antibiotics in ruminant feeds has been banned, supplementing fibrous forages with probiotics that can survive in the rumen has become a substitute. If these microbes can colonize and establishes (gain stability) in the rumen, then fibrolytic enzyme availability would be continuous. This could probably be a better approach compared to other available feed additives. In ruminant nutrition direct-fed microbial (DFM)

* Corresponding author. Tel.: +27 72 138 0604; fax: +27 33 260 5067.
E-mail addresses: Fonf@ukzn.ac.za, fabiannde@yahoo.co.uk (F.N. Fon).

0921-4488/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.smallrumres.2012.09.016

have been applied to maximize forage utilization and enhance production efficiency. Milk production, average daily weight gain, dry matter intake, microbial population, fibre utilization and animal performance have shown marked increased upon supplementation with DFM (Aydin et al., 2009). However, Raeth-Knight et al. (2007) did not observed any significant change in their digestibility parameters when supplemented with DFM. The novelty of this study is the application of crude microbial consortia (containing bacteria, protozoa and fungi) for inoculation compared to pure and specific microbial strains often utilized. In a previous *in vito* study, 11 herbivores microbial ecosystems were scanned in search of potential fibrolytic ecosystems of which horse (H), wildebeest (WB), zebra (ZB) and elephant showed the highest enzyme specific activities. Therefore, investigating the *in vivo* effect of DFM consortia from H, WB and ZB on ruminal fermentation is essential.

2. Materials and methods

2.1. Faeces collection, inocula (direct-fed microbials) preparation and culturing

For microbial inoculum preparation, samples (faeces) were collected in winter from horses (from University of KwaZulu-Natal research farm grazing on *Pennisetum clandestinum* (Kikuyu grass) standing hay and supplemented with veld hay while in paddocks), wildebeests and zebras (from Tala Game Reserve, KZN, SA, grazing on a dry land in an open field where Kikuyu standing hay and other grass hay were dominant) with no preference to sex. Faeces were collected on farm within 2 min of defecation from horse (H), zebra (ZB) and wildebeest (WB) before transferring into an airtight thermo flask (38 °C) which had been flushed with CO_2. While in the laboratory, 300 g of faeces was mixed with 300 ml of warm incubation buffer (salivary buffer, Tilley and Terry, 1963) and bubbled with CO_2 before squeezing through four layers of cheese cloth to make faecal fluid (FF). Faecal fluid preparation was done within 5 min in order to minimize anaerobic microbes exposure to oxygen. Faecal inocula from H, WB and ZB were used to create two combined microbial consortia (CS); N1 (H + WB, 1:1) and N3 (WB + ZB, 1:1). These inocula (198 ml) were incubated with salivary buffer (402 ml) containing 6 g of MS (2 mm) and lucerne (LC, 2 mm) (1:1) for 72 h at 38 °C. The activeness of these microbial consortia was determined by monitoring pH changes and gas pressure. After 72 h of incubation, the sample fluid for each consortium was used as direct-fed microbials for sheep inoculation.

2.2. Experimental animals, design, diet and composition

Six fistulated male Merimo sheep (from Ukulinga Research farm) with an average mass of 44 (SD = ± 1.5) kg were divided into two groups of three (control and treatment animals) separated by a distance of 20 m. These animals were housed in individual pens (230 cm × 203 cm) and fed at *ad libitum*. Each sheep was fed a total of 1.3 kg feed (maize stover and lucerne, 1:0.3) per day (0.8 kg and 0.5 kg at 8:00 and 15:30, respectively). Each sheep was given a 12 kg mineral block (of molasses base, from Voermol Feed Maidstone, KZN, SA) at the start of the experiment and residue weighed at the end of the trial. Water was provided *ad libitum*. Pens were shaded and properly ventilated. Animals were fed for a total of 21 days including 15 days of adaptation. All animals' treatments were governed by the UKZN ethical roles (Ethics number: 083/10/Animal).

The rationed feed was ground through a 2-mm sieve and DM determined after drying for 24 h at 60 °C. The micro-Kjeldahl method was used to determine nitrogen and crude protein (CP) calculated as N × 6.25 according to AOAC (Ref. S585.A8) (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest et al. (1991) using the ANKOM Technology Method. Hemicellulose was the difference between NDF and ADF while the difference between ADF and ADL was cellulose. The feed (935 kg DM) chemical composition was 723, 447, 60, 276, 386 and 87 NDF, ADF, ADL, hemicellulose, cellulose and crude protein respectively.

2.3. Sheep inoculation, in sacco degradability and ruminal fermentation parameters

In experiment 1, three of the six fistulated sheep were dosed (through the fistula) with 50 g of fresh faecal matter from N1 while the other three received no treatment (control). After 3.5 days, each treatment sheep was dosed again with 50 ml of cultured N1. All inocula (N1) were cultured in the laboratory for a maximum of 3.5 days. This implies that after every seventh day of the experiment, fresh samples were collected for inoculation and culturing for subsequent inoculation (after 3.5 days). After adaptation period, sheep dosing continued at the same interval with the same mass or volume till the end of experiment 1. Because of the limitation of fistulated animals only one treatment was tested at a time (3 control and 3 treatment sheep). In the second trial (experiment 2), three sheep were dosed with N3 while the other animals received no treatment (control). In experiment 2, the feeding process, inoculation, adaptation, sample incubation and collection were the same as in experiment 1.

In sacco dry matter degradability (DMD) of MS was determined by incubating approximately 3.0 g (ground through a 2 mm sieve) in nylon bags (41 μm pore size; bag size 6.5 cm × 14 cm) in the rumen of fistulated sheep (Kempton, 1980). Sequential addition method was adopted for sampling with a maximum number of 10 bags per sheep. All bags were withdrawn from the rumen after incubating for 96, 72, 48, 24, 12, 9, 6 and 3 h. Incubated bags were thoroughly washed under running tap water until no coloured liquid could be extruded before transferring into a washing machine (Hoovermatic, model T4350, South Africa) for a final wash. Incubated bags were washed (6 min × 5 min) together with three nylon bags (containing 3.0 g of MS) that were not incubated in the rumen to determine the washing losses. After washing, bags were dried for 2 days at 60 °C, cooled in a desiccator and weighed. The degradability at each time interval was calculated by taking the mean value obtained from the set of bags as well as for the 0 h. For each incubation time, one bag (two for 24 and 48 h) was incubated in each animal for each treatment (control, N1 or N3) that was replicated thrice per treatment. The degradability (Y) of MS at time (t) was estimated by using the non-linear curve modified by Dhanoa (1988):

$$Y = A + B(1 - e - C * (t - \mathrm{lt})), \quad \mathrm{PD} = A + B, \quad \mathrm{ED} = \frac{A + B * C}{C + 0.03}$$

where Y is the disappearance of DM at time (t); A is the readily soluble fraction of MS (washing loss); B is the insoluble but degradable fraction of MS; C is the rate of degradation of B; lt is the lag time; PD is the potential degradability; ED is the effective degradability and 0.03 h^{-1} is the assumed passage rate (kp) as determined by Nsahlai et al. (1998).

For pH measurements, rumen fluid (RF) was collected from each sheep on day 20 at the following times: 0, 3, 6, 9, 12 and 21 h. The pH of samples was determined on-farm after which 5 ml was acidified in 1 ml of 25% (v/v) metaphosphoric acid solution (to stop microbial activity) and stored in ice for short chain fatty acid (SCFA) analysis. Samples were stored in ice for a minimum of 30 min (to precipitate contaminating proteins) or in the freezer until when required for analysis. After centrifugation (8000 × *g*, 20 min at 4 °C), the filtrate (2 ml) was filtered through a syringe filter (0.45 μm pore from SUPELCO, USA) into a 2 ml clear ABC Screw Top Vial from SUPELCO, USA. Vials were then transferred into an automatic sampler (HT280 from Brescia, Italy) linked to a Gas Chromatograph (YL6100GC, Young Lin, Korea) for analysis. A temperature-programmed cycle from 90 to 120 °C rising by 5 °C was applied in the column (BPX 70 × 30 × 0.25 × 0.25). The injector block temperature was maintained at 260 °C with nitrogen as the carrier gas (60 ml/min). Hydrogen and air flow rate to the detector (flame ionization detector) were 60 and 300 ml/min, respectively. The injection volume was 1 μl/split 1:80. Molar concentrations of SCFA were determined from a linear curve of standards (acetic acid, propionic acid, *n*- and iso-butyric acid and valeric acid) that were run together with the samples. For pH and SCFA measurements, RF was collected once for each animal at different time intervals per treatment that was replicated thrice. Theoretical calculations of CO_2 and methane (CH_4) were estimated as described by Groot et al. (1998) based on the stoichiometric balance of fermentation of glucose to propionic acid, acetic acid, butyric acid, iso-butyric acid, CO_2 and methane (Czerkawski, 1978).