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Using single or multiple liquor-donor cows for in vitro digestibility of amylase- and sodium sulfite-treated neutral detergent fiber with ash correction

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ABSTRACT

In vitro methods requiring ruminal microorganisms to ferment and digest feeds have been used for decades. Though commonly accepted, collecting and pooling rumen fluid from different donor animals to avoid individual characteristics could affect in vitro fermentations. The current study evaluated the effects of individual or pooled liquors on in vitro digestibility of amylase- and sodium sulfite-treated NDF with ash correction (aNDFom). The study was conducted on 24 samples (8 alfalfa hays, 8 grass hays, and 8 corn silages). The 3 donor animals (treatment 1, 2, and 3) were selected based on similar body weights, parity, days in milk, milk production, and milk composition. Samples were digested in vitro via inoculation of different rumen fluid at different time points (12, 24, 72, and 120 h). An equal amount of each liquor collected was sampled and equally mixed with the others to obtain treatment 4. For the alfalfa hay group, differences were observed at 12 (29.95, 27.07, 29.02, and 32.55% aNDFom for treatments 1, 2, 3, and 4, respectively) and 24 h (37.35, 35.54, 36.44, and 40.56% aNDFom for treatments 1, 2, 3, and 4, respectively). The inoculum source did not affect in vitro digestibility over longer time periods (72 and 120 h). Similar results were observed in the grass hay group, in which the mixed inoculum had greater digestibility values at both 12 (28.86, 26.89, 27.88, and 30.92% aNDFom for treatments 1, 2, 3, and 4, respectively) and 24 h (37.35, 35.54, 36.44, and 40.56% aNDFom for treatment 1, 2, 3, and 4, respectively), but not over longer time periods. For the corn silage group, we observed differences for treatment 4 only at 12 h (35.78, 33.87, 34.83, and 37.80% aNDFom for treatment 1, 2, 3, and 4, respectively). These results underline the differences among donor animals, especially when evaluating short incubation time points, and that pooling rumen contents is not equal to averaging across individual animals. Reported data require a deeper investigation on whether or not the method of inoculating a pool of rumen contents represents the actual ability of the animal to digest fiber.
Key words: rumen inoculum, in vitro fermentation, amylase-treated ash-corrected neutral detergent fiber with addition of sodium sulfite digestibility

INTRODUCTION

In vitro methods requiring rumen inoculum to ferment and digest feeds have been used for decades (Tilley and Terry, 1963; Goering and Van Soest 1970). In particular, in vitro digestibility of NDF (**IVNDFD**) can be correlated with animal performance (Allen and Oba, 1998) and used in rationing programs (Fox et al., 2004; Van Amburgh et al., 2015). The accuracy of these in vitro procedures is affected by different factors (Church and Petersen, 1960; Ayres, 1991), including the way samples are dried and ground (Wilman and Adesogan, 2000; Damiran et al., 2008). Others factors are related to the procedures (Uden et al., 1974; Marinucci et al., 1992; Holden, 1999) or to the dietary forage source of the rumen content-donor cow (Bezeau, 1965; Jung and Varel, 1988; Cherney et al., 1993; Soder, 2005).

Recently, a ring test has been conducted to evaluate the variability of in vitro digestibility techniques among different laboratories (Hall and Mertens, 2012); this study also reported the methods used by participating labs to evaluate digestibility. The majority of these laboratories collected and pooled rumen content from different cows, but there were differences in number of donor cows used, stage of lactation, and diet. Collecting rumen content from more than one donor cow is done frequently (Krizsan and Huhtanen, 2013; Palmonari et al., 2014, 2016), but, to our knowledge, no trials have been conducted to evaluate the effects of one or more donor cows on IVNDFD. The objective of the current study was to evaluate the possible effects of a single or mixed ruminal inoculum on IVNDFD of 3 different forage types.

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MATERIALS AND METHODS

The study was conducted at the University of Bologna and all procedures that included animals were approved by the University of Bologna Institutional Animal Care and Use Committee.

Sample Description

The alfalfa hay group (*Medicago sativa*, 8 different samples) was composed of different cuts (mostly second and third). Grass hay (8 unique samples) was mainly composed of different cool-season grasses; briefly, the pool of grass hay included wheat (*Triticum aestivum*), Italian ryegrass (*Lolium multiflorum*), and wild oats (*Avena fatua*). The corn silage group (*Zea mays,* 8 samples) was representative of some of the main hybrids commonly used for animal feedstuffs in Italy. Samples were treated as described in a previous paper (Palmonari et al., 2016). Briefly, forages were dried in a forced-air oven (55°C) for 48 h and ground through a 1-mm screen in a cyclone mill (model SM100, Retsch, Haan, Germany). The samples were analyzed for CP (AOAC 976.06, 984.13), amylase-treated ash-corrected neutral detergent fiber with addition of Sodium Sulfite (**aNDFom**; Mertens, 2002), ADF (AOAC, 1990; method 973.18), and ADL (AOAC, 1990; method 973.18), as described in a previous paper (Palmonari et al., 2016).

Donor Cows and Rumen Fluid

Three lactating Holstein cows were selected as donors based on similar BW, parity, DIM, milk production, and milk composition (SCC, fat and protein, lactose, and urea; Table 1). Animals were milked twice a day. Donor cows were fed a hay-based diet (Table 2) containing alfalfa hay (42% aNDFom), grass hay (48% aNDFom), and corn grain (62% starch). Corn grain was ground with an industrial mill to obtain a final average meal size of 1.2 mm. Rumen fluid was sampled via esophageal probe, pouring off the first volume collected to avoid saliva or mucous contamination, and immediately placed in a thermostatic bottle. Sampling was conducted 3 h after feeding. Rumen contents were filtered through 4 layers of cheesecloth under constant O_2-free CO_2. The 3 donor animals were designated as treatment 1, 2, and 3. Once filtered, an equal volume of each liquor collected was sampled and equally mixed with the others to obtain the fourth treatment.

IVNDFD

In vitro fermentations for NDF digestibility were performed at 12, 24, 72, and 120 h using the Tilley and Terry modified technique (Tilley and Terry, 1963; Robertson and Van Soest, 1981), and according to the procedure described by Palmonari et al. (2016). Briefly, 10 mL of rumen fluid were added to each 150-mL Erlenmeyer flask that had been placed in a heated (39.3°C) water bath under CO_2-positive pressure to ensure anaerobiosis. Then 0.5 g of ground sample was weighed into each flask before the addition of 40 mL of buffer as described by Goering and Van Soest (1970). Each sample was digested in duplicate, in each of the 2 different in vitro incubations run, for a total of 4 replicates per sample. The 2 fermentations both started within 6 d of each other. For all analyses sample preparation was the same, as were the donor cows and their diet. At the end of the fermentation, contents of each flask were analyzed to determine NDF content of the residue and filtered through crucibles (40 μm porosity) with the addition of microfiber glass filters (1.5 μm, Whatman, GE Healthcare, Pittsburgh, PA). Residues were then treated following the procedure described by Goering

Table 1. Characteristics and production of rumen content donor cows

	Treatment[1]		
Measure	1	2	3
BW, kg	648	655	651
Lactation number	2	2	2
DIM	121	109	118
Milk production, kg/d	36.3	35.8	36.2
Milk composition, %			
Fat	3.48	3.50	3.47
Protein	3.31	3.38	3.33
Lactose	5.02	4.96	4.85
SCC, $\times 10^3$/mL	137	148	145
Urea, mg/100 mL	12.8	16.3	15.7

[1]Treatments 1, 2, and 3 represent the individual donor animals.

Table 2. Composition of diet fed to rumen fluid donor cows

Diet composition	% of diet DM
Ingredient	
Grass hay	25
Alfalfa hay, 1st cut	25
Corn grain, finely ground	25
Barley grain, flaked	10
Soybean meal, 48% CP	10
Cane molasses	2.5
Chemical composition	
aNDFom[1]	33.1
ADF	21.5
ADL	3.2
CP	14.8
Ether extract	3.09
Ash	9.05
Starch	22.17

[1]aNDFom = amylase- and sodium sulfite-treated NDF with ash correction.