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Short communication

Effects of monensin on the chemical composition of the liquid associated microbial fraction in an *in vitro* rumen fermentation system

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ABSTRACT

The hypothesis of this study was that monensin alters chemical composition of liquid associated rumen microbes. Furthermore, this study generates information on the chemical composition of rumen bacteria as affected by a ionophore by analyzing different parameters within the same experiment. Monensin at 5 and 8 μM was incubated with 380 mg of substrate (hay:concentrate 70:30 w/w) for 24 h in an *in vitro* gas production system. Monensin elicited no effects on nitrogen and true protein contents of the liquid associated microbial fraction, but sugar content and carbon did change on addition on monensin. Ratio of crude protein to purine bases decreased on adding monensin. True dry matter digestibility and short chain fatty acids production decreased on addition of monensin. Acetate, butyrate and valerate proportions decreased, whereas propionate and branched-chain fatty acid proportions increased. The change in acetate to propionate ratio concurs with the increase in the efficiency of microbial protein synthesis and the decrease in methane production. Results suggest that: monensin influenced the chemical composition of the liquid associated microbial fraction *in vitro*, and the changed ratio of crude protein to purine bases may lead to under- or over-estimations of microbial protein synthesis when purine derivatives are used as markers in the presence of monensin.

1. Introduction

Monensin is a ionophore compound with reported capability to change the rumen fermentation. Increased milk production, decreased methanogenesis and attenuation of certain cattle digestive disorders are some of the benefits attributed to monensin (McGuffey et al., 2001; Russell, 1996). These changes are associated with the property of ionophores to alter the microbial populations in the rumen: Gram-positive, proteolytic and obligate amino acid fermenting bacteria are sensitive to ionophores; while, gram-negative bacteria are not sensitive to them (Russell, 1996). Rumen microbes contribute substantially to the nutrients reaching the small intestine. Therefore, information on the chemical composition of the rumen microbes is essential for better understanding the nutrition of the animals. Previous studies, especially during the 1970s and early 1980s, have reported contents of N, amino acids, nucleic acids, carbohydrates, lipids and ash (*e.g.* Smith and McAllan, 1974; Storm and Ørskov, 1983) and more recently also on purines

Abbreviations: BCFA, branched-chain fatty acids; CP, crude protein; EMPS, efficiency of microbial protein synthesis; NDS, neutral detergent solution; PB, purine bases; PF, partitioning factor; SCFA, short chain fatty acids; TDMD, true dry matter digestibility; TP, true protein

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(Rodríguez et al., 2000) in rumen microbial fraction. It is clear that some of these components vary with factors such as diet and time after feeding (Smith and McAllan, 1974) but less clear is how the chemical composition of the microbial fraction varies with the inclusion of additives in the diet. Therefore the objective of this study was to add information on the changes of the chemical composition of the liquid associated microbial fraction (LAM) as affected by monensin. The hypothesis of this study was that the addition of monensin changes the chemical composition of LAM in an *in vitro* fermentation system.

2. Materials and methods

2.1. Methods

2.1.1. In vitro gas production method

2.1.1.1. Rumen fluid. The fistulation of the cows and the regular collection of rumen fluid were approved on the 30 June 2009 by the regional council of Stuttgart, Germany under the experiment number A 355/09 TE. The rumen fluid was collected before the morning feeding from two rumen-fistulated Holstein-Friesian cattle, one fed *ad libitum* on grass silage, and the other on a concentrate containing diet (concentrate 2 kg/day and grass silage *ad libitum*). The concentrate (in g/kg) comprised of barley (350), wheat (340), maize (100), soybean meal (170), soybean oil (10) and mineral mixture (30). The cows had free access to drinking water. The collected rumen fluid was brought to the laboratory in warm (39 °C) insulated flasks, both fluids were mixed together, homogenized and filtered through 100 μm nylon filter. The glassware used was continuously flushed with CO_2, stirred and kept in a water bath at 39 °C.

2.1.1.2. Method. The *in vitro* Hohenheim gas test (HFT) was used according to the protocol of Makkar et al. (1995) based on the method of Menke et al. (1979), except that double strength buffer and 380 mg of substrate were used. A mixture of hay and concentrate (70:30 w/w) was used as the substrate incubated in 100-ml-capacity calibrated glass syringes having 30 ml of a buffered incubation medium containing rumen microbes.

Just before dispensing the buffered rumen fluid, a freshly prepared monensin (sodium salt 90–95% TLC; Sigma-Aldrich, Stenheim, Germany) aqueous solution was injected into the syringes through the syringe nozzle to reach the desired concentration of 5 and 8 μM in 30 ml of the medium. These concentrations were decided from previous experiments in our laboratory were monensin applied at a concentration of 5 μM decreased gas production without decreasing short chain fatty acids (SCFA) production (Selje-Assmann et al., 2008). The set without monensin containing 380 mg of the substrate was used as a control. A corresponding blank consisted of the buffered medium without the substrate but containing monensin at the experimental concentrations. The buffered medium was dispensed into the syringes and the incubation was performed in a water bath at 39 °C for 24 h.

On a single day, each set of treatments comprised of ten syringes: two syringes for the blank and eight syringes for the treatment. From these eight syringes, six were used to collect the microbial fraction (see Section 2.1.2) and two were used to perform the protozoa count. Each experiment was repeated on three different days ($n=3$). The experimental design used was completely randomized with three replications for each treatment.

After incubation for 24 h gas, methane, protozoa number, true dry matter digestibility (TDMD) and SCFA were determined as described in Castro-Montoya et al. (2011). Partitioning factor (PF) and microbial mass production were calculated as described by Blümmel et al. (1997). The true dry matter digestibility (TDMD) was calculated as the difference between original sample weight (on dry matter basis) and residue after neutral detergent solution treatment and then divided by the original sample weight (on a dry matter basis).

2.1.2. Collection of microbial fraction from the liquid associated microbes

After 24 h of incubation, the contents of six syringes were filtered through a previously weighed nylon bag (pores size of 25 μm, F57, ANKOM Technology, NY, USA). The filtrate containing microbes was received in a tube, which afterwards was centrifuged at 17,000*g* at 4 °C for 20 min. The supernatant was removed and the microbial fraction was washed by adding 30 ml distilled water. Centrifugation was repeated and the microbial fraction was lyophilized, ground using a ball mill (Retsch, MM200, Haan, Germany) for 2 min at a frequency of 30 /s and stored at room temperature.

2.1.3. Chemical composition of the liquid associated microbial fraction

Reducing sugar content was determined using the method of Hodge and Hofreiter (1962) and expressed as glucose equivalent; purine bases (adenine and guanine) by the method of Makkar and Becker (1999); and microbial protein content by Lowry's method (Peterson, 1983). For N and C analyses 50 mg of microbial fraction were weighed into a crucible in duplicate and placed on the C/N analyzer system (Vario Max CN Elementar Analysensysteme GmbH. Frankfurt Main, Germany).

2.2. Statistical analysis

The main effects of monensin were tested by the GLM procedure of SAS software, according to

$$Y_i = \mu + \beta_{1i} + \xi_i$$

where μ is the overall mean, β_{1i} the effect of the *i*th treatment and ξ_I the error term. Significanct effects ($P<0.1$) of level of monensin were characterized using orthogonal contrasts testing the probability of linear or quadratic responses. Coefficients for polynomial contrasts were calculated for the unequally spaced treatments using the ILM procedure of SAS software. The values reported are means and standard errors.