Animal Feed Science and Technology 195 (2014) 38–46

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Effect of dietary fructose polymers or sucrose on microbial fermentation, enzyme activity, ciliate concentration and diversity of bacterial flora in the rumen of rams

Anna Kasperowicz[a], Katarzyna Stan-Głasek[a], Barbara Kowalik[a], Anna Vandzurova[b], Peter Pristas[b,*], Janusz Pająk[a,1], Elżbieta Kwiatkowska[a], Tadeusz Michałowski[a]

[a] The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jabłonna, Instytucka 3, Poland
[b] Institute of Animal Physiology, Slovak Academy of Science, Soltesovej 4, 040 01 Kosice, Slovakia

ARTICLE INFO

Article history:
Received 9 September 2013
Received in revised form 23 June 2014
Accepted 25 June 2014

Keywords:
Rumen
Fructans
Protozoa
Eubacteria
PCR-DGGE
Enzymatic activity

ABSTRACT

Five rumen-cannulated Polish Merino rams were used in a 5 × 5 Latin square design to investigate the effect of sucrose and polymers of fructose on the activity and characteristics of rumen protozoa and bacteria. The animals were fed concentrate and timothy hay containing either 33 g or 163 g fructan/kg. The low-fructan hay was supplied in the control diet and in the diets supplemented with sucrose and inulins. The high-fructan hay was the component of diet rich in timothy grass fructan. The control diet supplied 39.6 g fructan/day. The remaining diet offered 195.6 g of fructose-containing carbohydrates/day. The concentration of total SCFA was 7.2–9.5 mM/100 ml rumen fluid independently of diet and sampling time ($P>0.05$). The increase in sucrose or fructan contents lowered the proportions of acetate from 73.1 to 64.2 mM/100 mM ($P\leq0.002$) and increased those of propionate and butyrate from 16.4 to 21.5 ($P=0.003$), and from 9.8 to 15.6 mM/100 mM ($P\leq0.004$), respectively. The sampling time affected the proportions of propionate ($P=0.01$). The diets did not affect pH of rumen fluid except in the ration with timothy grass fructan ($P=0.029$).

Irrespective of diet and sampling time ($P\geq0.05$), the digestion velocities of timothy grass fructan, inulin, sucrose, starch, pectins, carboxymethylcellulose, and xylan were 1.3–2.9; 0.7–2.1; 4.8–9.6; 1.9–2.4, 0.3–0.5, 0.7–1.2, and 0.7–1.4 mM reducing sugars/g dry matter (DM) of rumen fluid per hour, respectively. A positive correlation was found between the degradation rate of timothy grass fructan and inulin or sucrose ($P\leq0.045$). The total number of ciliates was 261–475 × 10^3/ml, that of *Entodinium*, 231–439 × 10^3/ml. The concentrations of *Epidinium*, *Isotricha* and *Dasytricha* were 14–24 × 10^3/ml, 2.6–7.0 × 10^3/ml, and 3.2–8.1 × 10^3/ml, respectively ($P\geq0.05$). The *Diplodinium* counts varied from 1.4 to 3.1 × 10^3/ml in control animals to 4.0–7.3 × 10^3/ml in sheep fed the diet rich in timothy grass fructan and Beneo P95 ($P\leq0.008$). A postprandial decrease in the concentration of *Diplodiniuum* was found in animals fed the control ration and the rations rich in timothy

Abbreviations: SCFA, short-chain fatty acids; WSC, water-soluble carbohydrates; DM, dry matter; CP, crude protein; CF, crude fiber; CFat, crude fat; F, fructan; DP, degree of polymerization; SET buffer, 75 mM sodium chloride, 25 mM edetate disodium salt (EDTA), 20 mM Tris (Trizma base); SDS, sodium dodecyl sulphate; PCR-DGGE, polymerase chain reaction-denaturing gradient gel electrophoresis; UPGMA, Unweighted Pair Group Method with Arithmetic Mean; CMCase, carboxymethylcellulase.

* Corresponding author. Tel.: +421 556783120; fax: +421 557287842.
E-mail address: pristas@saske.sk (P. Pristas).

[1] Deceased.

http://dx.doi.org/10.1016/j.anifeedsci.2014.06.014

grass fructan and Beneo HPX (P=0.017). It was stated that the sampling time influenced the composition of bacterial flora. The experiment showed that the applied doses of the examined carbohydrates did not affect the majority of the quantified parameters. The studies shall be continued using enlarged doses of fructose-containing carbohydrates.

1. Introduction

Fructans are water-soluble storage carbohydrates found in many green plants fed to ruminants as forage. They consist of fructose residues linked either by β2,6(levans) or β2,1(inulins) glycosidic bonds. Usually, at the end of a polymer chain there is a molecule of glucose linked to a fructose unit by a β2,1-bond (Pollock and Chatterton, 1988). Sucrose exhibits the same chemical structure and, due to this, it can sometimes be considered as the core unit of fructans. This disaccharide is also often present in feeds given to ruminants.

Both sucrose and fructans can be digested in the rumen exclusively by autochthonous saccharolytic and fructanolytic microorganisms inhabiting this chamber of the complex stomach of ruminants (Merchen, 1988; Chesson and Forsberg, 1997). Thomas (1960) showed that the bacterium *Streptococcus bovis* possesses fructanolytic and saccharolytic abilities. Similar properties have been detected in strains of rumen treponemes (Kasperowicz et al., 2010a) and *Pseudobutyrivibrio ruminis* (Kasperowicz et al., 2010b; Stan-Głasek et al., 2010). Rumen protozoa, especially holotrichs, also exhibit the ability to utilize sucrose and fructans (Williams and Coleman, 1992). On the other hand, our *in vitro* studies showed that the density of cultured strains of bacteria depends on the kind of fructans supplied to the growth medium (Kasperowicz et al., 2010a, 2010b). No similar experiment has been performed *in vivo*. Some data concerning the influence of dietary water-soluble carbohydrates (WSC) on ruminal microorganisms reported, however, showing that supplying sucrose or sugar beet pulp and molasses to the diets for ruminants resulted in decreasing fibrolytic bacteria and/or activity of fibrolytic enzymes (Huhtanen and Khalili, 1992; Monsoni et al., 2007). Yáñez-Ruiz et al. (2006) showed that enrichment of a control diet with WSC enhanced the total number of protozoa. An increase in protozoa number in the rumen of red deer was also observed when they were fed fresh chicory (Hoskin et al., 1995).

It was found that supplementation of a forage diet with WSC affects the rumen environment through a reduction of pH and an increase of SCFA concentration, together with higher molar proportions of propionic and butyric acids at the expense of acetic acid, compared with a control diet (O'Mara et al., 1997; Lee et al., 2002, 2003). On the other hand, the rumen fermentation pattern depends on the type of WSC in the ration (Oba, 2011). There are, however, no available reports on the effect of different kinds of fructans and sucrose on the metabolic characteristics and microbial population in the rumen of the same host animals.

Taking the above into account, we designed the study presented in this report, aiming to evaluate the effect of a diet enriched with fructose-containing carbohydrates on the fermentation pattern, activity of carbohydrate-digesting enzymes, and differentiation of the populations of bacteria and ciliate fauna in the sheep rumen.

2. Materials and methods

2.1. Materials

Two timothy hays with different levels of fructans were used in the experiment. Timothy hay 1 contained 313.6 g/kg crude fiber (CF), 71.8 g/kg crude protein (CP), 13.8 g/kg crude fat (CFat), and 33 g/kg fructan (F), whereas timothy hay 2 had 231.5, 62.8, 17.5, and 163 g/kg of CF, CP, CFat, and F, respectively. Both timothy grasses were cultivated in Jablonna (Poland). Timothy hay 1 was harvested in May and timothy hay 2, in September 2009. The CF content was determined by the Weende method of Hennenberg and Sthomann (Tecator note AN-301) with the use of a Fibretec (Sweden), CP and CFat were assayed by the method of Kjeldahl and Soxlet, respectively, according to the notes from Tecator, with a Kjeltec or Soxtec apparatus (Sweden). The fructan content was determined spectrophotometrically at 480 nm by the method of Roe (Southgate, 1991) with fructose as the standard. One gram of dry ground timothy hay was washed twice with 100 ml of ethanol at a concentration of 860 ml/L to remove simple sugars and chlorophyll, decanted and dried. Then 1000 ml of distilled water was added and extraction was performed for 4 h under continuous mixing. The mixture was filtered through Whatmann No. 1 paper and 1 ml of extract was assayed for fructan content. Beneo HPX inulin consisted of 995 g/kg of 2,1-β-D-fructose polymers, the average degree of polymerization (DP) was 23. Beneo P95 inulin consisted of 932 g/kg of 2,1-β-D-oligosaccharides with DP ≤8 and 68 g/kg glucose, fructose, and sucrose. The inulins were purchased from Beneo-Orafti (Belgium). Sucrose was a commercial product bought at a supermarket.

Phosphates, carbon tetrachloride, and formaldehyde were supplied by Polish Chemical Reagents, Poland (Polskie Odczynniki Chemiczne, Polska). Cellulose and reagents for enzymatic assays, *i.e.*, dinitrosalicylic acid, pectin, carboxymethylcellulose, xylan, starch, chicory inulin, and sucrose were purchased from Sigma–Aldrich Chemicals. Timothy grass fructan was obtained in the laboratory according to Ziołecki et al. (1992).