Effect of Water-Soluble Browning Products in Heated Herbages on Rumen Microorganisms

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Abstract

When feed is heated, browning can occur. This browning is detrimental to both the value of feed and physiological functioning of the animal. Browning occurs when polymeric substances are produced during the Maillard reaction. Indigestible soluble Maillard reaction products could affect nitrogen utilization by ruminants. A method has been established for isolation of water-soluble browning products using a reversed phase column. In the present work, the effect of water-soluble browning products isolated from heated herbage on rumen microorganisms was investigated. A solution of browning products was added to the medium 10 to obtain final concentration of 0, 0.5 and 2.0 g/L and incubated. When glucose-glycine (GG) browning products and those extracted from perennial ryegrass or timothy was added, gas production yield increased dependent on the increasing levels of browning products. When GG or browning products extract from perennial ryegrass were added, the protein concentration increased in order 0.0 g/L < 0.5 g/L < 2.0 g/L. However when timothy-browning product was added, an increase in the protein concentration was not observed. This observed difference in protein concentration suggests that the influence of water-soluble browning products to the growth activity of rumen microorganisms depends on its origin.

Keywords: Heated herbage, Maillard reaction, rumen microorganisms, microorganism protein

Introduction

Heat treatment is used for improving the feed utilization and reducing the activity of nutritive inhibitors. When feed is heated, browning often occurs. The main causative factor of browning through heating is considered to be due to the Maillard reaction. The Maillard reaction occurs between carbonyl compounds and amino groups. In the early stage of the Maillard reaction, palatability and digestibility of feed are improved. However, in the later stages, brown polymeric products, melanoidin, are produced and the feed utilization decreases, especially in protein quality (Van Soest, 1982). Due to melanoidin´s undigestible properties, its effects have always been considered negatively in terms of evaluation of feed. However, Van Soest et. al. (1991) reported that indigestible soluble Maillard reaction products could affect nitrogen utilization in ruminants. While, nitrogen utilization has been implicated, a complete explanation or mechanism of how soluble Maillard reaction products affect the ruminant is not clear. Rumen microorganisms play an important role in the ruminant nitrogen utilization. Therefore, investigating the effects of water-soluble browning products on rumen microorganisms is significant to ruminant nutrition. In the present study, using isolation methods, we investigate the effect of water-soluble browning products isolated from heated forage on rumen microorganisms.

Material and Methods

Perennial ryegrass (*Lolium perenne* L.) and timothy (*Phleum pratense* L.) were grown as pure swards and cut at the booting stage. Each fresh herbage of the grasses was heated in an airtight can at 60 °C for 24 h and then extracted with 0.1M sodium acetate solutions with occasional stirring for 24 h at room temperature. The acetate solution was obtained by filtration and applied to a reversed phase cartridge column pre-washed with ethanol and water. Material retained by the cartridge column was washed with water and then

eluted with 20% ethanol. The eluate was concentrated in vacuum and lyophilized. Model Maillard browning products (GG) were prepared by heating a mixture of D-glucose and glycine (1M: 1M) dissolved in 0.1M sodium hydrogen carbonate solution at 95 °C for 24 h. The resulting brown solution was placed in cellulose tubing (MW>12000) and dialyzed against water. The nondialyzate was condensed *in vacuum* and lyophilized. The composition and preparation of the medium 10 without agar for rumen incubation followed the method of Caldwell & Bryant (1966). An ox with a permanent rumen fistula served as the source of inoculum. The ox was fed orchardgrass (*Dactylis glomerata*) hay *ad libitum*. The solid and liquid rumen materials, were collected before morning feeding. The rumen contents were well agitated and strained through two layers of cheesecloth, which was gassed with a heavy stream CO^2. All subsequent operations were conducted under anaerobic conditions. The strained content was then serially diluted, in 5-fold steps, in the anaerobic mineral solution of Bryant and Burkey (1953). 1 ml of dilution was inoculated via pipette into vials of modified medium 10 (30ml). Then solution of browning products (1ml) was added to the media to obtain final concentration of 0, 0.5 and 2.0 g/L. All experiment vials were incubated at 39 °C for 48 h. After incubation for 24 h or 48 h, pH, gas production and protein content were determined. Protein contents were determined using the Bio-Rad DC Protein Assay kit (Bio-Rad, No.500-0112).

Results and discussion

Samples pH was not influenced by the kind of browning products, incubation time and addition levels. pH ranged from 6.58 to 7.21. The effects of browning products on gas production are shown in table 1. GG or browning products extracted from perennial ryegrass resulted in increase of both gas production and protein concentration in the medium. This same tendency is observed when products from timothy were added. It can be seen from the table when GG and browning products provided from perennial ryegrass were added, the

largest increase in gas production occurred when the largest quantity of product was present. However this pattern was not observed in timothy (when timothy = 0.5 g/l, gas production = 13.0 ml, when timothy = 2.0 g/l, gas production = 13.4 ml). Gas production was almost equal when both 0.5 g/l and 2.0 g/l of timothy were present in the medium. Figure 1 describes the observed pattern of increase in protein concentration in response to quantity of browning product. The same pattern of increase in rumen protein concentration is seen for GG and perennial ryegrass extract only. These results suggest that the addition of GG and browning products provided from perennial ryegrass increase the rumen microorganism's activity. The difference observed in gas production and protein concentration between browning products provided from perennial ryegrass and timothy suggests that the influence of water-soluble browning products on rumen microorganism's activity is dependent on the origin of water-soluble browning products.

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Table 1 – Gas production yield in media incubated for 24h with browning products.

Additional level	GG	Rye	Timothy
0.0 g / l	8.5	10.7	10.7
0.5 g / l	9.5	12.8	13.0
2.0 g / l	11.0	14.4	13.4

GG: Water-soluble browning products provided from glucose-glycine
Rye: Water-soluble browning products provided from perennial ryegrass
Timothy: Water-soluble browning products provided from timothy

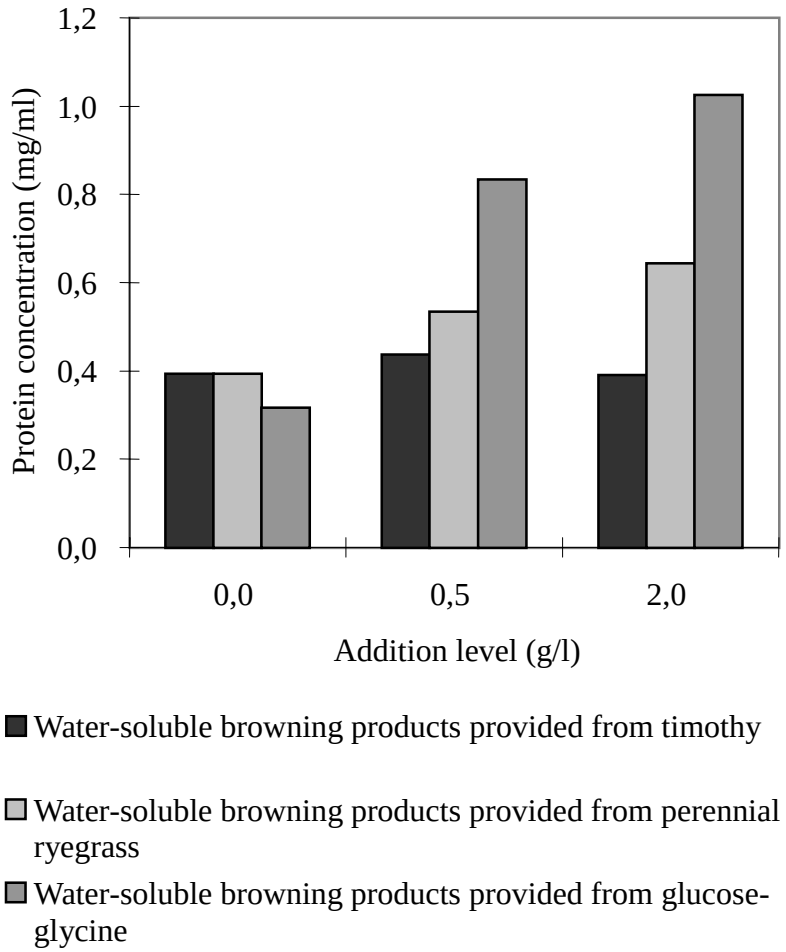

Figure 1 - Protein concentration in media incubated for 24h with browning products.

E. Touno, Fig1.