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Oral presentation

Determination the effects of capsaicin on the growth of pure cultures of rumen bacteria

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Abstract

Capsaicin that is a major pungent component of red pepper is widely used as food additive and considered to be an antimicrobial factor. In this study, it was aimed to determine the effects of capsaicin on the growth of pure cultures of Gram-positive and Gram-negative rumen bacteria to evaluate potential of capsaicin as an alternative to ionophore antibiotics in modification of ruminal fermentation. The antibacterial activity assays of capsaicin were carried out using broth microdilution method under strictly anaerobic conditions inside an anaerobic chamber. Capsaicin was used in a dose range of 0.5-256 µg/mL. Capsaicin exhibited potential antibacterial activity on *Ruminococcus flavefaciens* and *Methanobacterium formicicum* ($p<0.05$), although it did not inhibit these bacteria completely. On the other hand, capsaicin showed growth stimulatory effect on Ruminococcus albus at 0.5-128 µg/mL doses ($p<0.05$), while potential antibacterial activity was observed at 256 µg/mL ($p<0.05$). Growth of other Gram-positive rumen bacteria, *Butyrivibrio fibrisolvens* and *Eubacterium ruminantium* were stimulated by capsaicin at 0.5-64 µg/mL and 8-128 µg/mL doses, respectively ($p<0.05$), however stimulatory effects disappeared at higher concentrations. Capsaicin had stimulatory effects on *Streptococcus bovis* from Gram-positive bacteria at all used doses ($p<0.05$). Capsaicin also showed stimulatory effects on the growth of Gram-negative rumen bacteria, *Megasphaera elsdenii* and *Fibrobacter succinogenes*, at 0.5-128 µg/mL and 1-256 µg/mL concentrations, respectively ($p<0.05$). Stimulatory effects of capsaicin on some hydrogen, formate and lactate producer Gram-positive rumen bacteria suggested that the mechanism of action of capsaicin in the rumen may be different from ionophore antibiotics.

Keywords: Antibacterial, capsaicin, rumen bacteria

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INTRODUCTION

Ionophore antibiotics have been used since 1970s in order to avoid unwanted ruminal losses and control metabolic disorders. But, use of antibiotics as feed additives was banned in European Union (EU) by January 21, 2006 since they leave residues in animal products and develop resistance in bacteria (Jouany and Morgavi 2007). An intense interest has occurred on safer antimicrobial agents which can be alternatives to antibiotics as feed additives after this ban. Mostly plant extracts and secondary bioactive plant metabolites are focused due to their potential to modify ruminal fermentation in recent years.

Capsaicin is a major pungent component of red pepper (Surh and Lee, 1996). It is also the main component of capsicum oil (10 to 15%) (Cichewicz and Thorpe, 1996). Capsaicin is widely used as food additive and considered to be an antimicrobial factor. Capsaicin was reported to have a strong inhibitory effect on Bacillus subtilis, Escherichia coli (Molina-Torres et al., 1999), Salmonella typhimurium, Pseudomonas aeruginosa (Careaga et al. 2003), Staphylococcus aureus (Omolo et al., 2014), Streptococcus pyogenes (Marini et al., 2015), and Helicobacter pylori (Jones et al., 1997). There are some reports on the effects of capsaicin on in vitro (Cardozo et al., 2004; Busquet et al., 2005) and in vivo (Cardozo et al., 2006) ruminal fermentation. However, to the our knowledge, the effects of capsaicin on pure cultures of rumen bacteria have not been evaluated previously. Such an information can contribute to the clear physiological mechanisms and the mode of action of capsaicin in the rumen.

Therefore, the objective of the present study was to investigate the effects of capsaicin on pure cultures of some Gram-positive and Gram-negative rumen bacteria.

MATERIAL AND METHODS

Capsaicin: Capsaicin was purchased from Santa Cruz Biotechnology (Istanbul, Turkey).

Bacterial strains: The Gram-positive bacterial species used in antimicrobial tests were Ruminococcus albus (ATCC 27210) and Ruminococcus flavefaciens Sijpestejin C97 (ATCC 49949) as hydrogen, formate and, acetate producers, Butyrivibrio fibrisolvens D1 (ATCC 19171) and Eubacterium ruminantium GA 195 (ATCC 17233) as butyrate producers, and Streptococcus bovis (ATCC 33317) as a lactate producer. Methanobacterium formicicum (ATCC 33274), a mesophilic methanogen, was used as a methane producer. The Gram-negative bacterial species tested were Fibrobacter succinogenes S85 (ATCC 19169) and Megasphaera elsdenii LC1 (ATCC 25940), which were used as succinate and propionate producers.

Anaerobic media: Growth media for bacterial cultures were prepared under CO2 to maintain anaerobic conditions according to Orpin (22). The chemical composition of anaerobic media is shown in Table 1. The media was gassed with CO2 while heating to 60 °C in a hot water bath to remove O2 completely. The conversion of the color of medium to dull yellow from bluish purple by the resazurin (0.1%, v/v), which is a redox potential indicator in the medium, was considered to be a sign of removal of oxygen. Bottle of media was closed with a rubber stopper and autoclaved. Anaerobic bacteria were grown at 37 oC for 24-72 h under strictly anaerobic conditions inside an anaerobic chamber (Whitley DG250, Don Whitley, West Yorkshire, UK) under an atmosphere of N2-CO2-H2 (80:10:10).

Antibacterial activity assays: The antibacterial activity assays of capsaicin were carried out using a broth microdilution method following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2016) in the anaerobic chamber. Stock solution of capsaicin (100 mg/mL) was prepared dissolving capsaicin in 50 % (v/v) ethanol. A serial 2-fold dilution of capsaicin (256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 µg/mL) was prepared in the anaerobic media. Two hundred microliters of each concentration was added to wells of a 96-well plate (Corning 3599, Flat bottom). Then, 20 µL aliquots of 4 × 1010 cell/mL bacteria were added into each well. Triplicate wells were used for each concentration. Negative control wells without antimicrobial compounds and media control wells without bacteria were maintained for each set. After incubation at 37 °C for 24 h in the anaerobic chamber, microbial growth was determined at 600 nm using a plate reader (BioTek, Epoch). A significantly lower OD600 value compared to control dose (0 µg/mL) was accepted as potential antibacterial activity (Ko et al., 2018) while significantly higher OD600 value was accepted as stimulatory activity.

Statistical analyses: Statistical analysis was carried out by the use of one-way ANOVA followed by Dunnett's test. Each well of a 96-well plate was an experimental unit. A value of $p<0.05$ was taken to indicate a significant difference.

RESULTS

Effects of capsaicin on rumen bacteria are presented in Figure 1 and Figure 2. Capsaicin exhibited potential antibacterial activity on R. flavefaciens and M. formicicum ($p<0.05$), although it did not inhibit these bacteria completely. On the other hand, capsaicin showed growth stimulatory effect on R. albus at 0.5-128 µg/mL doses ($p<0.05$), while potential antibacterial activity was observed at 256 µg/mL ($p<0.05$). Growth of other Gram-positive rumen bacteria, B. fibrisolvens and E. ruminantium were stimulated by capsaicin at 0.5-64 µg/mL and 8-128 µg/mL doses, respectively ($p<0.05$), however stimulatory effects disappeared at higher concentrations. Capsaicin had stimulatory effects on Streptococcus bovis from Gram-positive bacteria at all used doses ($p<0.05$). Capsaicin also showed stimulatory effects on the growth of Gram-negative rumen bacteria, M. elsdenii and F. succinogenes, at 0.5-128 µg/mL and 1-256 µg/mL concentrations, respectively ($p<0.05$).

DISCUSSION

In this study, capsaicin was evaluated for its potential to be an alternative to ionophore antibiotics in modification of ruminal fermentation. Capsaicin had a potential antimicrobial activity on R. flavefaciens at all concentrations and on R. albus at only highest concentration. Ruminococcus species produce mostly hydrogen, formate and, acetate in the rumen. Capsicum also had potential to inhibit methane producing M. formiciccum, at doses above 4 µg/mL. On the other hand, capsicum stimulated the growth of butyrate, and propionate producing bacteria in this study. Calsamiglia et al. (2007) reported that capsaicin may increase propionate production, and reduce acetate or methane production. Fandi˜no et al. (2008) also reported that capsicum increased butyrate proportion from 13.0 to 14.1 mol/100 mol, and reduced acetate proportion from 55.3 to 54.0 mol/100 mol versus control. The results of these studies are consistent with the results of our study. Stimulatory effects of capsaicin on butyrate producing bacteria suggested that the mechanism of action of capsaicin in the rumen may be different from ionophore antibiotics. Nevertheless, the stimulatory activity of capsaicin on some acetate and butyrate producer Gram-positive bacteria like R. albus, B. fibrisolvens and E. ruminantium disappeared at higher concentrations in the present study. Some phytochemicals could promote in vitro bacterial growth and feed utilization in the rumen at low doses while they exhibited inhibition at high doses (Demirtas et al., 2019; Patra et al., 2012). Therefore, further studies are required on the effects of higher doses of capsaicin on pure cultures of rumen bacteria to clarify the mode of action of capsaicin on rumen fermentation.

CONCLUSIONS

Stimulatory effects of capsaicin on some acetate, butyrate and lactate producer Gram-positive rumen bacteria especially in low doses suggested that the mechanism of action of capsaicin in the rumen may be different from ionophore antibiotics. However, further studies are required on the effects of higher doses of capsaicin on pure cultures of rumen bacteria to clarify the mode of action of capsaicin on rumen fermentation.

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