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Effect of β-carotene on selected indices of *in vitro* rumen fermentation in goats*

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ABSTRACT

Three goats fitted with rumen fistula were used as donors of rumen fluid. Substrates were incubated *in vitro* with buffered rumen fluid for 24 h. Different levels of β-carotene (five groups: 10, 50, 100, 200, 500 mg/l) were added as five treatment groups to determine whether it affected rumen fermentation *in vitro* as compared with blank samples used as controls. NH_3-N concentrations with 50, 100 and 200 mg β-carotene/l group were found significantly lower than that in blank samples ($P<0.01$). Microbial protein concentrations in all β-carotene-added groups were higher than in blanks, while those found in 200 and 500 mg/l groups were significantly higher ($P<0.05$ and 0.01, respectively). Total VFAs seemed not to be affected by added β-carotene ($P>0.05$), although propionate and butyrate concentrations changed. It is concluded that β-carotene can enhance the utilization of NH_3-N by rumen microorganisms and thus promote their growth *in vitro*.

KEY WORDS: β-carotene, rumen fermentation, goat, *in vitro*

INTRODUCTION

Healthy food has attracted much attention, and many researchers have devoted studies on improving the value of milk by effectively transferring nutrients to milk from feed. It has been shown that carotenoids can be transferred to milk from feedstuff. Despite the large variety of carotenoids in plants, no more than 10 are found in ruminant feeds, and the most quantitatively important are β-carotene and lutein. Since the β-carotene content in feedstuff is limited, adding commercial products is effective. Little attention has been paid to the effect of β-carotene on rumen fermentation and its disappearance in the rumen. The first event in the digestive process of carotenoids is degradation of the vegetable matrix that releases carotenoids into the rumen liquid phase (Mora et al., 1999). The extent of carotenoid degradation by microorganisms

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in the rumen remains uncertain because of the wide range of results from *in vitro* and *in vivo* studies, mostly on β-carotene. Whereas some authors reported no degradation (Dawson and Hemington, 1974; Cohen Fernandez et al., 1976a), others found moderate (10-25%; Davison and Seo, 1963; Potkański et al., 1974; Cohen Fernandez et al., 1976b; Mora et al., 1999) or higher β-carotene disappearance (40-55%; King et al., 1962). In the experiment of Hino et al. (1992) β-carotene could greatly alleviate inhibition by grass lipids, mainly galactolipids and phospholipids, of the growth of rumen microorganisms and fibre digestion, and could stimulate the growth of cellulolytic bacteria. The reason for this effect is unclear (Hino et al., 1992). In the previous experiment, the effect of β-carotene on rumen fermentation was seldom studied *in vitro*. Therefore, the objectives of this study were: 1. to observe the effect of β-carotene on selected rumen fermentation indicators, 2. to estimate whether β-carotene at a high concentration (500 mg/l) was safe for rumen microorganisms, and 3. to validate that β-carotene can stimulate the growth of rumen bacteria.

MATERIAL AND METHODS

Design and treatments

Three goats fitted with rumen fistula were used as donors of rumen fluid. The diet components are given in Table 1 and the substrates were the same. Goats were fed two times per day for at least 15 d prior to sampling. Ruminal fluid was collected *via* the cannula and squeezed through two layers of cheesecloth. Filtered ruminal fluid (1000 ml) was mixed with 4000 ml of manufactured saliva (Menke and Steingass, 1988). The manufactured saliva was maintained at 39°C during preparation. Each *in vitro* flask contained 20 ml of the inoculum and 40 ml man-made saliva

Table 1. Ingredients and chemical composition of diets, % of DM

Item	
Ingredient,%	
broken rice	33.6
soyabean meal	14
grass hay	51
minerals	1.4
Chemical composition	
DE, MJ/kg	11.29
CP, %	13
Ca, %	0.52
P, %	0.36
NDF, %	41.75

and 1 g of air-dried substrate, five doses of β-carotene (Roche Switzerland, 10% CWS) were added to each flask as five treatments and one blank served as the control. The content in each treatment was 0, 10, 50, 100, 200, 500 mg/l in six groups (blank, groups A, B, C, D and E, respectively). Flasks were purged with CO_2, sealed with vented stoppers, and incubated in an environmental shaker (100 rpm) at 39°C. Substrates for the incubations were similar to the diets fed to donor goats (Table 1). Feedstuffs were ground through a 1-mm screen. Substrates were incubated for 0, 1, 3, 5, 8, 12 and 24 h. Blanks for each goat and each time point were run concurrently. Each incubation was conducted in triplicate (separate runs) for a total of 126 flasks [six β-carotene treatments (including the blank) × three triplicates × seven time points]. One flask was considered an experimental unit.

Samplings, recordings and analysis

At the end of each incubation time, the flasks were swirled vigorously, and then three 5-ml aliquots of the contents were removed using a wide-tip pipette, one sample used for determining the ammonia nitrogen (NH_3-N) concentration and the others were frozen (-20°C) until analysis for microbial protein concentration (MCP) and VFA.

Statistical analyses

The effects of β-carotene on NH_3-N, MCP, VFA were analysed by analysis of variance with treatments. Data were analysed within each time point (0, 1, 3, 5, 8, 12 and 24 h) between groups, and the mean value of each group also analysed. All statistical analyses were done using SAS (1996).

RESULTS

Changes of NH_3-N concentration during 24 h incubation were similar among groups, tended to be elevated at the beginning and then dropped. The ranges of NH_3-N concentrations in 24 h were, mg/100 ml: blank 24.03-36.70; group A 18.70-27.99; group B 12.38-27.52; group C 16.37-27.55; group D 14.38-27.40 and group E 28.69-33.64. The average values of each group were 30.65, 23.52, 20.31, 22.45, 20.22 and 29.84 mg/100 ml. In the blank, the NH_3-N concentration reached the peak value, while all treated groups showed a downward trend. NH_3-N concentrations in all treated groups were lower than the blank between 3 to 24 h. After 24 h fermentation, the concentration in the 200 mg/l group was lower than all other groups.

MCP concentrations in the treatment groups were higher than the blank in the incubation, and the MCP concentration increased as the dose of β-carotene rose, especially the 500 mg/l group was the highest at 5 h compared with the other groups ($P<0.01$), but was lower than in the 200 mg/l group later. The ranges of MCP concentration in 24 h were, mg/ml: blank 0.024-0.099; group A 0.037-0.117; group B 0.027-0.121; group C 0.034-0.101; group D 0.047-0.117; group E 0.049-0.117. The average values of each group were 0.055, 0.057, 0.076, 0.024, 0.097 and 0.100 mg/ml.

Changes of the acetic acid to propionic acid ratio were similar in the groups before 8 h incubation, while at 12 and 24 h, the acetic acid concentrations in the 10, 50, 100 and 200 mg/l groups were higher than in the blank, and at the 24 h-point the differences were significant ($P<0.01$). The ranges of acetic acid concentrations at 24 h were, mmol/l: blank 32.04-127.94; group A 33.02-115.07; group B 37.63-123.57; group C 37.79-117.37; group D 27.50-121.10; group E 53.86-96.82. The average values of each group were 67.45, 66.92, 78.97, 70.94, 76.88 and 74.99 mmol/l. The ranges of propionic acid concentrations at 24 h were, mmol/l: blank 5.63-75.25; group A 5.58-40.38; group B 12.15-33.77; group C 6.44-36.68; group D 5.02-40.85; group E 8.93-57.56. The average values of each group were 25.86, 19.57, 21.36, 18.76, 22.27 and 26.47 mmol/l.

Total VFA concentrations in the groups were similar 24 h ($P>0.05$), but in the 500 and 200 mg/l groups they reached the highest values, while at 5 h and 8 h, the differences were significant in relation to the blank ($P<0.01$). The ranges of TVFA concentrations at 24 h were, mmol/l: blank 40.76-217.09; group A 41.60-176.38; group B 53.28-180.19; group C 47.83-173.90; group D 35.15-183.15; group E 68.12-189.97. The average values of each group were 96.28, 96.45, 110.31, 99.44, 111.27 and 115.56 mmol/l.

DISCUSSION

The effect of β-carotene on rumen fermentation has seldom been reported. Some studies reported that β-carotene plus α-tocopherol enhanced bacterial cell yield in the presence of safflower oil, caprate, stearate, or linoleate in incubation *in vitro*, suggesting that β-carotene and α-tocopherol increase the utilization of fatty acids. β-carotene plus α-tocopherol also stimulated cellulose digestion in the presence of 100 mg/l of safflower oil, evidently through the increased growth of cellulolytic bacteria. The decrease of NH_3-N concentrations in the treatment groups and the increase of MCP compared with the blank may have resulted from β-carotene stimulating the growth of cellulolytic bacteria, but the optimal concentration of β-carotene was not certain, maybe between 200 and 500 mg/l. The increase in the acetic acid concentration in TVFA may indicate that β-carotene can change the

type of rumen fermentation and this was significant in dairy production in dairy cows. Compared with β-carotene, α-tocopherol was more effective in increasing the growth of rumen bacteria, but it inhibited growth at high concentrations, proving that added β-carotene in ruminant feeds was safe and effective (Hino et al., 1992).

CONCLUSIONS

In this study, we concluded that β-carotene can improve the utilization of NH_3-N and the production of microbial protein concentration, maybe due to the increased the growth of cellulolytic bacteria; the 500 mg/l dose of β-carotene did not inhibit rumen fermentation, suggesting that adding β-carotene to ruminant feed was safe; β-carotene may have changed the acetic acid and propionic acid concentrations in rumen fermentation, but did not influence TVFA production. The mechanism of β-carotene action is still unclear and should be studied more *in vivo*.

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