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The effect of different doses of *Astragalus* root extract on *in vitro* rumen fermentation of steam-flaked maize grains used as an only substrate*

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ABSTRACT

An *in vitro* study was conducted to determine the effect of different doses of *Astragalus* root extract *(*ARE, Huangqi) on rumen fermentation of steam-flaked maize grains. Rumen fluid was taken from 3 crossbred steers fed a 55% forage to 45% mixed concentrate ratio diet. As ARE dose was increased, ruminal ammonia-N concentration was decreased in a dose dependent manner, whereas total volatile fatty acid concentration and total gas production were increased linearly (P<0.001). Increasing ARE doses resulted in decreased molar proportion of acetate and increased molar proportion of propionate as well as a decrease in acetate: propionate ratio. In conclusion, ARE could stimulate the mixed ruminal fermentation as well as modify the pattern of rumen fermentation *in vitro.*

KEY WORDS: plant extract, rumen fermentation, *in vitro* gas production

INTRODUCTION

Steam-flaked maize is a widely used grain for feeding beef and dairy cattle in many countries, because steam flaking can effectively improve the feeding value of grains, principally by increasing starch digestibility in the rumen and total tract (Zinn et al., 1995). However, with starch gelatinization degree of steam-flaked maize grains increased, the starch is more available to ruminal degradation, resulting in a more rapid rate of ruminal fermentation and accumulation of organic acid within the rumen.

Feeding antibiotics to ruminant animals for keeping their rumen fermentation balance is a common feeding practice in many countries. However, due to residues

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and resistant strains of bacteria, and increasing awareness of hazards associated with antibiotics, the use of antibiotics in animal feeds are reduced. Thus, there are increasing interests in exploiting natural products that have no public health hazards.

The natural products, including organic acids, plant extracts and enzymes, have great attractions for the public. Martin and Streeter (1995) reported that DL-malate stimulated the *in vitro* ruminal fermentation by increasing production of propionate, total VFA and final ruminal pH. Other acids including L-aspartate, fumarate, and L-malate may elicit the activity of ruminal bacteria. Plant extracts have been used for centuries for various purposes (as traditional medicine and food preservatives, mainly for human consumption) due to their antimicrobial properties. Using 12 plant extracts and 6 secondary plant metabolites, Busquet et al. (2006) revealed that most plant extracts and secondary plant metabolites influenced rumen fermentation characteristics, implying their functions in the manipulation of ruminal microbial fermentation.

Astragalus root extract (ARE) is a traditional Chinese medicinal herb. It contains multiple bioactive components, including polysaccharides, saponins, flavones and others, which have been demonstrated to succeed in increasing the production of animals, such as swine and poultry, and reducing the suffering for illness (Liu and Zhao, 2002). However, there is little information available regarding the effect of ARE on rumen microbial fermentation characteristics. The objective of the present study was to evaluate the effect of different doses of ARE on *in vitro* rumen fermentation of steam-flaked maize grains used as an only substrate.

MATERIAL AND METHODS

Steam-flaked maize grains from Hebei Kaite Biological Technology Corporation, Xingtai Districtiion, Hebei Province (China), were used as a sole fermentation substrate. Steam-flaked maize grains were grounded by a high-speed grinder and passed through a 1-mm screen automatically.

The astragalus extract (ARE) product was purchased from a local commercial pharmaceutical company, which was a crude extract from the root of *Radix astragali* extracted with ethanol. The product was labeled to contain some active components including flavones, polysaccharides, alkalines, saponins and trace mineral elements. The doses of ARE supplementation were: control (no addition), 25, 50, 75 and 100 mg/l mixed culture fluid, respectively.

Short-term *in vitro* gas production incubations were carried out with rumen fluid from 3 ruminally fistulated crossbred steers (1/2 Simmental, 1/2 Native Yellow cattle, BW = 350 kg) fed a 55:45 forage: concentrate diet twice daily (08.00 and 17.00), 3 of Chinese wild rye-grass, 2 kg lucerne pellets and 4 kg mixed concentrate supplement (consisting of, %: ground maize 63, soyabean meal

16, soy hulls 19, limestone 0.9, calcium phosphate 0.5, salt 0.5, mineral-vitamin mixture 0.1). Before rumen contents were obtained, the 3 steers had been fed the above diet for 7 days for adaptation. The steers were cared under the approval of the China Agricultural University, Animal Science and Technology College, Animal Care and Use Committee.

The rumen contents were thoroughly mixed and strained through 4 layers of cheese-cloth, then mixed in a 1:1 proportion with a buffer solution used by Martin and Streeter (1995). The steam-flaked maize grains samples (300 mg, DM basis) were weighed into each of three calibrated glass syringes with 100 ml calibrated volume (Häberle Maschinenfabrik GmbH, Germany). A total of 0, 250, 500, 750 and 1000 mg of ARE were separately dissolved in 100 ml of distilled water, and 0.3 ml of each stock solution was added into each of syringes (30 ml culture fluid) to achieve final concentrations of 0, 25, 50, 75 and 100 mg/l, respectively. The syringes were prewarmed to 39°C, then 30 ml of mixed culture fluid (consisting of ruminal fluid and buffer, ratio 1:2) were pipetted with an automatic pump into each glass syringe followed by incubation in a water bath at 39°C. The gas production was measured and recorded as the volumes of gas in the calibrated syringe at the incubation of 24 and 72 h. The control incubation (non ARE added) was carried out with the equivalent amount of distilled water (0.3 ml). The same fermentation was repeated on 2 separate days. At 24 h of incubation, the fermentation was stopped by placing the syringes into ice-cooled water.

The pH of the culture fluid was measured immediately with a pH meter (Model PHS-3C, Leici, Shanghai). The fermentation contents were centrifuged at 6,000 *g*. One milliliters supernatant fluid from each centrifugal tube was taken to determine volatile fatty acid (VFA) and ammonia N concentration. The sediment pellets were determined for DM digestibility. Ammonia N was determined according the method of Broderick and Kang (1980). VFA was analysed by gas chromatography (SP-3420, Beifen Ruili Analytical Equipment Co., Beijing) equipped with a Flame Ionization detector (FID), using a PEG-20M+H_3PO_4 glass column of 2 m × 6 mm × 2 mm in size. The supernatant fluid samples (0.6 μ) were injected with a special syringe (maximal scale: 1 ul), and the temperature of the injector/detector and the column was set at 260 and 220°C, respectively. Nitrogen was used as a carrier and a 30 ml/min gas flow rate.

All statistical analyses were conducted using SAS (1996). Differences between treatments and control were declared significant at $P<0.05$ using the Dunncan comparison test.

RESULTS AND DISCUSSION

As shown in Table 1, total gas production of 24 and 72 h increased in a linear manner (L; $P<0.01$; $P<0.03$) when the supplemental levels of ARE increased from

0 to 100 mg/l. The maximum gas productions with increasing supplemental levels of ARE followed the similar way. In contrast, the pH values of fermentation fluid decreased in a linear manner (L; P<0.0001); at the higher doses of 75 and 100 mg/l, they were significantly lower than those of control (Table 2). Furthermore, *in vitro* ruminal DM digestibilities increased (P<0.03) in a dose-dependent manner when supplemental levels of ARE increased from 50 to 100 mg/l.

Table 1. Effect of different doses of ARE (ml) on dynamic traits of *in vitro* gas production of steam-flaked maize grains, ml/0.3 g DM

Items	ARE dose, mg/l						P	
	0	25	50	75	100	SEM[1]	L[2]	Q[2]
24 h gas production	78.7	81.4	82.0	86.2	86.5	1.905	0.014	0.490
72 h gas production	83.9	87.2	89.4	91.4	92.6	2.144	0.030	0.798
maximum gas production	83.7	86.7	88.1	90.1	90.9	2.134	0.064	0.721
Rate of gas production, h	0.134[b]	0.139[ab]	0.143[ab]	0.146[ab]	0.149[a]	0.003	0.003	0.685
Lag time, h	0.945[a]	0.661[b]	0.481[c]	0.416[c]	0.389[c]	0.040	<0.0001	0.0019

[1] SEM - standard error of the mean; [2] L - linear effect due to dose of ARE; Q - quadratic effect due to dose of ARE; [3] [a,b,c] means with different superscripts in the same line differ significantly (P<0.05)

With increasing supplemental levels of ARE, a linear decreased manner (P<0.0001) of ammonia-N concentration was observed. Furthermore, relative to control, the ARE supplemental levels of 75 or 100 mg/l reduced ammonia-N concentration (P<0.001). As the supplemental levels of ARE increased, the decreased ammonia N concentration is in line with the increased gas production and reduced ruminal pH values discussed above, suggesting increased synthesis of rumen microbial proteins as a result of more fermentation of steamed-flaked maize grains stimulated by ARE in this study.

As shown in Table 2, total VFA concentration increased (P<0.0001) in a linear manner; at the doses of 50, 75 and 100 mg/l, total VFA concentrations were significantly higher (P<0.05) than those of 0 and 25 mg/l doses. On one hand, steamed-flaked maize grains were used as an only substrate in this study and would be easily fermented by ruminal microbes. On the other hand, ARE contains some kinds of polysaccharides, such as glucan and heteropolysaccharides, which are also ready fermentable carbohydrates and stimulate rumen fermentation activities, allowing more steam-flaked maize grains to be fermented. Therefore, as the doses of ARE increased, the increased gas production, total VFA and the decreased pH values are expected.

As to individual VFA molar proportions, increasing the supplemental dose of ARE resulted in increased proportions of propionate and butyrate as well as valerate (P<0.05), but decreased proportions of acetate. In addition, increasing the

ARE dose resulted in a linear reduction of iso-butyrate and iso-valerate (P<0.05). This observation implies that feeding astragalus plant extract may be nutritionally beneficial for rumen fermentation and the performance of ruminant animals fed on steam-flaked maize grain-based diets.

Table 2. Effect of different doses of ARE on the fermentation of steam-flaked maize grains by mixed ruminal microorganisms *in vitro* after 24 h incubation

Item	ARE dose, mg/l					SEM[1]	P	
	0	25	50	75	100		L[2]	Q[2]
pH	6.68[a]	6.65[a]	6.62[ab]	6.55[b]	6.53[b]	0.024	<0.001	0.928
Ammonia-N, mg/l	122.82[a]	116.45[a]	112.43[a]	80.02[b]	79.66[b]	6.83	<0.001	0.536
Total VFA, mM	95.22[b]	96.88[b]	103.16[a]	103.81[a]	104.39[a]	1.560	<0.001	*0.211*
Individual VFA molar proportion, mol %								
acetate	54.59[a]	53.99[ab]	52.38[b]	53.46[ab]	52.81[b]	0.388	0.007	0.107
propionate	27.85[b]	28.04[ab]	28.81[a]	28.47[ab]	28.37[ab]	0.172	0.022	0.026
iso-butyrate	0.66[a]	0.63[a]	0.58[b]	0.58[b]	0.56[b]	0.011	<0.0001	0.144
butyrate	14.8[c]	15.36[b]	16.32[a]	15.5[b]	16.25[a]	0.062	<0.0001	0.0005
iso-valerate	1.12[a]	1.01[ab]	0.95[ab]	1.01[ab]	0.96[b]	0.026	0.005	0.421
valerate	0.93[b]	0.97[ab]	0.96[ab]	0.99[ab]	1.05[a]	0.024	0.019	0.017
Acetate: propionate	1.96[a]	1.93[ab]	1.82[c]	1.87[bc]	1.86[bc]	0.022	0.005	0.037
DM digestibility, %	54.55[b]	55.45[b]	58.01[ab]	60.25[a]	60.44[a]	1.055	<0.001	0.571

[1] SEM - standard error of the mean; [2] L - linear effect due to dose of ARE; Q - quadratic effect due to dose of ARE; [3] [a,b,c] means with different superscripts in the same line differ significantly (P<0.05)

Busquet et al. (2006) found that, supplementation of fenugreek extract in the diet did not reduce the concentration of *in vitro* ruminal VFA, even at high doses, but increased the molar proportion of propionate, and decreased the molar proportion of BCFA (branched chain fatty acid) and ammonia N concentration. These results are consistent with the current results using ARE. Francis et al. (2002) suggested that the effectiveness of plant extracts could be attributed to the present of some plant saponins, which have an inhibitory effect on rumen protozoa. In addition, Wallace et al. (1994) and Francis et al. (2002) demonstrated that inhibitory effects of plant saponins on ruminal bacteria seem to be more pronounced against Gram-positive bacteria (similar to the action of ionophores), which are normally acetate, but not propionate producers**.** In this experiment, some saponin or saponin-like substances present in the astragalus plant extract may in part inhibit the activity of rumen protozoa and bacteria, resulting in accumulation of propionate and reduced molar proportion of acetate in the rumen. Further studies are necessary to determine the effectiveness of ARE products on *in vivo* rumen microbial fermentation and animal performance.

CONCLUSIONS

Increasing doses of astragalus plant extract linearly increased total rumen VFA production and molar proportion of propionate, but decreased the molar proportion of acetate and acetate to propionate ratio, implying that feeding astragalus plant extract may be nutritionally beneficial for rumen fermentation and the performance of ruminant animals fed on steam-flaked maize grain-based diets. The astragalus root extract (ARE) product, however, significantly decreased ruminal pH values, especially at 75 and 100 mg/l levels. Further studies are necessary to determine the effectiveness of ARE products on *in vivo* rumen microbial fermentation and animal performance.

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