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IN VITRO ALTERATIONS IN RUMINAL PARAMETERS BY *MEGASPHAERA ELSDENII* INOCULATION ON SUBACUTE RUMINAL ACIDOSIS (SARA)

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Abstract

SARA is a common and serious problem in well-managed and intensive dairy herds or beef cattle operations, because of triggering other metabolic disorders and causing lactation-fertility losses. It is a metabolic disease in dairy cattle that occurs during early and mid-lactation and has traditionally been characterized by low rumen pH, but lactic acid does not accumulate as in acute lactic acid acidosis. Managing the disease, rather than eliminating it, has been suggested in high-producing dairy herds. SARA was induced in vitro to appraise the effectiveness of Megasphaera elsdenii inoculation. Rumen fluid was collected from 2 ruminally cannulated Holstein heifers. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). The media was then added with a test diet consisting (g/kg) of 550-soluble starch, 260-glucose, 60-cellulose, 70-cellobiose and 60-tripticase, at levels of 10, 15, 20, 25, 30, 40, 50, 70 and 100 g/l. After determining its level causing SARA as reflected by pH (~5.8) in preliminary experimentation, the substrate (test diet, 25 g/l) were incubated with presence of 0, 10^5, 10^6, and 10^7 cfu M. elsdenii per ml at 39°C for 24 h. Rumen parameters were analyzed by 2-way ANOVA. It is determined that most of the tested parameters are not influenced after inoculation of M. elsdenii, but the level of NH3-N (P<0.002) and Butyric acid (P<0.006) in rumen fluid, are observed to increase with the growth of bacterium level. When the bacterium is inoculated in the level of 7 cfu ml^{-1}, it is reached to the highest level of butyric acid (20.29 mM). It is showed that the existent evidence is similar to other studies. In conclusion, addition of M. elsdenii into media, one of the predominant lactate-utilizing bacteria failed to reverse SARA in vitro.

Key words: *in vitro, subacute ruminal acidosis (SARA), Megasphaera elsdenii, rumen fermentation.*

INTRODUCTION

Subacute ruminal acidosis (SARA) is a common and serious problem in well-managed and intensive dairy herds or beef cattle operations, because of triggering other metabolic disorders and causing lactation-fertility losses. *Megasphera elsdenii* is one of the bacteria that are presented in the rumen of high-grain fed cattle (McDaniel et., 2009; Klieve et al., 2003). *Megasphaera elsdenii* is utilized by about 97% of lactate that was generated from starch fermentation (Counotte et al., 1981; Piknova et al., 2004). It is confirmed by both *in vitr*o and *in vivo* studies that rumen pH and acidity can be regulated by the increase in the population of lactate-utilizing bacteria like *M.elsdenii,* so that acidosis may be prevented (Greening et al., 1991; Robinson et al., 1992; Kung and Hession, 1995; Wiryawan and Broker, 1995; Henning et al., 2010). *M. elsdenii* is reported to reduce adaptation period by 5-7 days to high-grain diet when introduced gradually (Klieve et al., 2003).

This study is performed to determine the effect of *M. elsdenii* on the rumen fermentation at *in vitro* SARA conditions.

MATERIALS AND METHODS

Prior to morning feeding, rumen fluids were collected from 2 ruminally cannulated Holstein heifers. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). In pressure-resistant Pyrex tubes, different amounts (10, 15, 20, 25, 30, 40, 50, 70 and 100 g/l) of test diet (550 g soluble

starch + 260 g glucose + 60 g cellulose + 70 g cellobiose + 60 g tripticase), was mixed with 20 ml rumen fluid and 30 ml buffer at 39°C for 20 h. pH and lactic acid concentration were determined (Sung et al., 2004) to assess amounts of substrates necessary to induce acidosis *in vitro*. After determining its level causing SARA as reflected by pH (~5.8) in preliminary experimentation, the substrate (test diet, 25 g/l) was incubated with presence of 0, 10^5, 10^6, and 10^7cfu *M. elsdenii* per ml at 39°C for 24 h. Gas production, VFA, lactic acid, and NH_3-N, and pH were measured at 2, 4, 6, 8, 10, 12, and 24 h relative to incubation. Amount of gas was calculated based on pressure, which was determined by digital manometer (with sensitivity of 0.2%; Keller Leo 1, Switzerland), in 100 ml bottle (Lopez et al., 2007).

The linear model included the effect of substrate, day, and sampling time as well as their interaction in data analysis using one-was ANOVA (SPSS 16.0.0, 2007). Significance was declared at $P<0.05$.

RESULTS AND DISCUSSIONS

All rumen response variables are summarized in Table 1. When the results of variable concentrations of *M. elsdenii* inoculation subsequent to SARA that is provided by test diet was analyzed; it is observed that 3 different inoculated bacteria concentrations had no effect on pH, lactate accumulation and gas formation levels. It was determined that ammonia (N-NH_3) concentration of *in vitro* media was higher (8.05 mmol/l) with M. *elsdenii* inoculation at 7 cfu ml^{-1} level ($P<0.002$).

Although pH, N-NH_3, lactate accumulation and gas formation levels of the samples that were obtained at the 4, 8, 12 and 24 hours of incubation were altered ($P<0.0001$), no interaction was observed between different bacteria concentration levels and time for pH, ammonia concentration, lactate accumulation and gas formation levels.

It was detected that bacteria inoculation was effective on butyrate concentration ($P<0.006$), highest butyrate concentration level was obtained by the bacteria inoculation at 7 cfu/ml^{-1} level. It was observed that bacteria inoculation had no effect on other volatile fatty acids (VFA). Total VFA concentration varied due to time, total VFA concentration increased as the incubation time prolonged. There was no interaction between time and bacteria in terms of VFA parameters.

Different results may be obtained from *in vitro* studies in comparison with *in vivo* studies because fermentation end products don't be absorbed and accumulate in the media (Menke et al., 1979). When the results of three different bacteria inoculation dosage after SARA that was obtained by test diet was analyzed, it was observed that most of the analyzed parameters hadn't been affected by *M. elsdenii* inoculation. However, ammonia and butyrate concentration of ruminal fluid increased by bacteria concentration elevation.

Table 1. Responses of rumen parameters to addition of *M. elsdenii* into media containing test diet.

Trt	Response variables											
Bacteria cfu ml^{-1}	pH	N-NH_3 (mM)	Lactate (mM)	Gas (ml)	Ac (%)	Pr (%)	Bu (%)	Isobu (%)	Va (%)	Isova (%)	ΣVFA (mM)	Ac:Pr
0	5.81	6.70	0.37	240.00	57.74	18.83	17.94	1.38	2.55	1.57	1.29	3.12
5	5.78	6.76	0.36	238.90	57.68	18.06	17.77	1.45	3.45	1.67	1.31	4.07
6	5.77	7.26	0.32	245.94	58.51	17.53	18.54	1.28	2.59	1.55	1.42	3.39
7	5.81	8.05	0.38	244.23	56.67	17.40	20.29	1.35	2.72	1.56	1.33	3.34
SEM	0.07	0.37	0.08	13.39	0.62	0.46	0.55	0.24	0.36	0.13	0.06	0.39
ANOVA												
B	0.9	0.002	0.36	0.97	0.26	0.1	0.006	0.97	0.27	0.93	0.41	0.38
T	0.0001	0.0001	0.0001	0.0001	0.13	0.78	0.17	0.89	0.08	0.61	0.0001	0.37
B*T	1	0.87	0.85	1	0.52	0.43	0.77	0.31	0.5	0.45	0.34	0.24

Trt: Treatment, B: Bacteria, T: Time

It is reported that inoculation of *M. elsdenii* to the *in vitro* fermentation media had reduced propionate formation and increased butyrate levels (Marounek et al., 1989; Slyter et al., 1992; Kung et al., 1995). Correlatively, bacteria inoculation caused an increase in butyrate formation in this study, too.

As a result of amino acid deamination that *Megasphaera elsdenii* participates in (Bladen et al., 1961; Russell et al., 1988; Rychlik et al., 2002), sometimes more ammonia than bacteria can utilize may be generated in rumen (Leng and Nolan, 1984). Accordingly, it is determined by *in vitro* experiment that *M. elsdenii* inoculation is effective on ammonia level ($P<0.002$).

CONCLUSIONS

M. elsdenii inoculation did not affect fermentation parameters in media containing test diet. Different bacteria levels had no effect on pH, lactate accumulation and gas formation levels; this is considered to be a result of unfavorable conditions for bacteria due to fermentation end products accumulation in media because of not being absorbed.

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