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ALTERATION IN RUMINAL FERMENTATION: THE EFFECT OF *MEGASPHAERA ELSDENII* INOCULATION ON SUBACUTE RUMINAL ACIDOSIS (SARA) *IN VITRO*

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Abstract

SARA is a serious herd problem in intensive dairy and beef operations because of triggering other metabolic disorders and causing lactation-fertility losses. SARA was induced in vitro to evaluate 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 were then added with 1) a test diet consisting (g/kg) of 550-soluble starch, 260-glucose, 60-cellulose, 70-cellobiose and 60-tripticase, 2) ground wheat and 3) ground corn, at levels of 10, 15, 20, 25, 30, 40, 50, 70 and 100 g/l. After determining their levels causing SARA as reflected by pH (~5.3) in preliminary experimentation, the substrates (test diet, 40 g/l; wheat, 30 g/l; corn, 50 g/l) were incubated with presence of 0, 10^5, 10^7, and 10^9 cfu M. elsdenii per ml at 39°C for 24 h. Rumen parameters were analyzed by 2-way ANOVA. There was substrate, but not inoculum level and substrate by inoculum interaction effects on measurements. The data confirm that increasing level of starch-rich feedstuffs leads to acidosis as reflected by decreased pH and the Ac:Pr ratio and increased lactate concentration. However, addition of M. elsdenii into media, one of the predominant lactate-utilizing bacteria failed to reverse SARA in vitro.

Keywords*: subactute ruminal acidosis, Megasphaera elsdenii inoculation, rumen fermentation, in vitro.*

INTRODUCTION

Introducing high energy diet after parturition as well as low absorptive capacity of rumen epithelium increase risk for subacute ruminal acidosis (SARA). About 97% of lactate resulting from starch fermentation is utilized by *Megasphaera elsdenii* (Piknova et al., 2004). Both *in vivo* and *in vitro* studies ascertained critical role of *M. elsdenii* in preventing lactic acid accumulation during transition to high-grain diet (Greening et al., 1991; Kung and Hession, 1995). *M. elsdenii* is reported to reduce adaptation period by 5-7 d to high-grain diet when introduced gradually (Klieve et al., 2003).

Kung and Hession (1995) reported that inoculation of 10^5 and 10 *M. elsdenii* cfu per ml *in vitro* elevated pH and reduced lactate concentration as compared to non-inoculation. They also reported that lactate concentration was <5mM during incubation with *M. elsdenii*. Greening et al. (1991) also showed that *M. elsdenii* inoculation increased pH and decreased lactic acid concentration in beef subjected to experimentally induced acidosis. Lactic acid-utilizing bacteria (*M. elsdenii* and *Selenomonas ruminantium*), alone or in combination alleviates adverse effect of rapidly fermentable carbohydrate introduction by slowing down pH reduction and lactate accumulation (Nocek et al., 2002; Wiryawan and Brooker, 1995). This experiment was carried out to demonstrate the effect of *M. elsdenii* on rumen fermentation characteristics in SARA *in vitro*.

MATERIALS AND METHODS

Prior to morning feeding rumen fluids were collected from 2 ruminally cannulated Holstein heifers. In pressure-resistant Pyrex tubes, test diet (550 g soluble starch + 260 g glucose + 60 g cellulose + 70 g cellobiose +60 g tripticase),

wheat, and corn were mixed at different amounts in 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*.

Test (40 g/l), wheat (30 g/l), and corn (50 g/l) diets were mixed with 20 ml rumen fluid and 30 ml buffer. Then, media were added with 0, 10^5, 10^7, and 10^9 cfu/ml *M. elsdenii*. 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. Using Real-Time PCR *S. bovis* and *M. elsdenii* were counted. 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 (SAS, 2002). Significance was declared at $P<0.10$.

RESULTS AND DISCUSSIONS

All rumen response variables are summarized in Table 1. pH decreased in test diet, whereas remained unchanged in wheat and corn diets. Lactic acid concentration was lower for test diet than for others. In SARA lactate-utilizing bacteria convert it to VFA (Nagaraja and Chengappa, 1998). Lactic acid concentration is 0.17-0.74 m*M* in SARA (Chıquette, 2009; Fulton et al., 1979, Oetzel et al., 1999; Plaizier et al., 1999). That is, there was no lactic acid accumulation in media. There was *M. elsdenii* inoculation effect on pH and lactic acid concentration.

NH_3-N concentration was the highest in wheat diet, and it was not affected by *M. elsdenii* inoculation. Gas production varied by the substrate, but not *M. elsdenii* inoculation. Substrate type affected total VFA and VFA profiles. However, *M. elsdenii* inoculation did not alter fermentation profile. Media containing wheat and corn diets as substrates had the highest number of *S. bovis* and *M. elsdenii*, respectively.

Table 1. Responses of rumen parameters to addition of *M. elsdenii* (10^{power}/ml) into media containing different substrates.

Trt*		Response variables										
S	I	*M. elsdenii* (log/ml)	*S.* bovis (log/ml)	pH	N-NH_3 (m*M*)	Lactate (m*M*)	Gas (ml)	Ac (%)	Pr (%)	Bu (%)	ΣVFA (m*M*)	Ac:Pr
TD		8.88^{ab}	9.49^{b}	5.93^{b}	7.2^{b}	0.90^{a}	50.3^{c}	32.0^{a}	30.4^{a}	21.1^{b}	192^{b}	1.19^{b}
WD		8.70^{b}	11.43^{a}	6.14^{a}	10.3^{a}	0.69^{b}	63.2^{a}	28.2^{b}	27.5^{b}	29.0^{a}	224^{a}	1.05^{b}
CD		9.18^{a}	9.12^{b}	6.15^{a}	7.4^{b}	0.57^{c}	60.8^{b}	31.9^{a}	26.1^{b}	28.6^{a}	211^{a}	1.47^{a}
	0	8.86	9.87	6.10	8.2	0.70	58.4	30.5	27.9	26.5	220	1.14
	5	8.96	10.21	6.10	8.6	0.71	59.7	30.2	28.0	27.2	210	1.23
	7	8.88	10.03	6.11	8.6	0.69	59.2	30.8	27.5	26.6	207	1.30
	9	8.90	9.97	6.09	8.5	0.68	59.8	30.6	27.1	27.8	209	1.32
TD	0	9.08	8.60	5.95	7.0	0.90	50.5	32.5	30.5	20.8	196	1.09
	5	8.52	9.98	5.93	7.3	0.90	50.5	31.6	30.3	21.0	189	1.08
	7	9.03	9.72	5.91	7.3	0.89	50.1	33.2	31.0	20.8	191	1.10
	9	8.93	9.60	5.93	7.4	0.93	50.0	30.6	29.8	21.9	193	1.50
WD	0	8.51	11.31	6.12	10.2	0.70	61.1	27.3	28.3	28.7	251	1.03
	5	9.16	11.53	6.16	10.5	0.71	65.3	28.0	27.3	29.5	221	1.03
	7	8.32	11.20	6.15	10.4	0.69	62.8	28.0	26.8	28.8	213	1.06
	9	8.74	11.72	6.15	10.2	0.64	63.5	29.4	27.6	29.1	211	1.09
CD	0	9.04	9.60	6.17	7.0	0.58	60.2	32.3	26.1	27.7	205	1.27
	5	9.16	8.89	6.15	7.6	0.58	59.5	31.7	27.4	28.6	210	1.53
	7	9.34	9.17	6.18	7.6	0.56	61.2	32.0	25.9	28.1	211	1.66
	9	9.14	8.84	6.12	7.6	0.57	62.0	31.7	25.0	30.0	216	1.43
SEM		0.26	0.39	0.12	0.4	0.06	8.1	1.1	1.0	1.4	15	0.13
Effect		----------					*P* > F	----------				
S		0.04	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.004	0.002
I		0.99	0.84	0.82	0.36	0.94	0.82	0.78	0.72	0.23	0.61	0.55
S x I		0.12	0.24	0.23	0.96	0.93	0.72	0.30	0.72	0.91	0.39	0.67

*Trt = treatment; S = substrate; I = inoculant.

TD = test diet, 40 g/l (550 g soluble starch + 260 g glucose + 60 g cellulose + 70 g cellobiose +60 g tripticase)/kg. WD = wheat diet (30 g/l), CD = corn diet (50 g/l).

CONCLUSIONS

Wheat favored *S. bovis* growth and corn favored growth *Elsdenii* growth. Both feedstuffs did not affect medium pH and had low lactic acid concentration in medium as compared to test mixture. *M. elsdenii* inoculation did not affect fermentation parameters in media containing test diet, wheat diet, and corn diet.

REFERENCES

Chiquette J., 2009. Evaluation of the protective effect of probiotics fed to dairy cows during a subacute ruminal acidosis challenge., Anim. Feed Sci. Technol.153, p.3-4, 278-291.

Greening R.C., Smolenski W.J., Bell R.L., Barsuhn K., Johson M.M., Robinson J.A., 1991. Effect of inoculation of Megasphaera elsdenii strain 407A (UC 12497) on ruminal pH and organic acids in beef cattle, J. Anim. Sci., 69 (Suppl. 1) 518 (Abstr.).

Fulton W.R., Klopfenstein T.J., Britton R.A., 1979. Adaptation to high concentrate diets by beef cattle. II. Effect of ruminal pH alteration on rumen fermentation and voluntary intake of wheat diets, J. Anim. Sci., 49, p.785-789.

Klieve A.V., Hennessy D., Ouwerkerk D., Forster R.J., Mackie R.I., Attwood G.T., 2003. Establishing populations of Megasphaera elsdenii YE 34 and Butyrivibrio fibrisolvens YE 44 in the rumen of cattle fed high grain diets, J. Appl. Microbiol., 95, p.621-630.

Kung L., Hession A.O., 1995. Preventing in vitro lactate accumulation in ruminal fermentations by inoculation with Megasphaera elsdenii, Anim. Sci., 73, p.250-256.

Lopez S., Dhanoa M.S., Dijkstra J., Bannink A., Kebreab E., France J., 2007. Some methodological and analytical considerations regarding application of the gas production technique. Anim. Feed Sci. Technol., 135, p.139–156.

Nagaraja T.G., Chengappa M.M., 1998. Liver abscesses in feedlot cattle: a review, J. Anim. Sci. 76, p.287–298.

Nocek J.E., Alman J.G., Kautz W.P., 2002. Evaluation of an indwelling ruminal probe methodology and effect of grain level on diurnal pH variation in dairy cattle, J. Dairy Sci., 85, p.422–428.

Oetzel G.R., Nordlund K.V., Garett E.F., 1999. Effect of ruminal pH and stage of lactation on ruminal lactate concentration in dairy cows, J. Dairy Sci., 82 (Suppl. 1), P35.

Piknova M., Filova M., Javorsky P., Pristas P., 2004. Different restriction and modification phenotypes in ruminal lactate-utilizing bacteria, FEMS Microbiology Letters 236, p.91–95.

Plaizier J.C., Martin A., Duffield T., Bagg R., Dick P., Mcbride B.W., 1999. Monitoring acidosis in the transition cow, J. Dairy Sci. 82, 110 (Abstract).

SAS, 2002. User's guide: Statistics. Version 9th, Statistical Analysis System, SAS Inst., Inc., Cary, NC.

Sung H.G., Bae H.D., Lee J.H., Kim D.K., Shin H.T., 2004. Application of LDH enzyme and viable LDH-producing bacteria to prevent lactate accumulation during in vitro rumen fermentation, Anim. Feed Sci. Technol., 117, p.235-243.

Wiryawan K.G., Broker J.D., 1995. Probiotic control of lactate accumulation in acutely grain-fed sheep, Aust. J. Agric. Res., 46, p.1555-1568.