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EFFECT OF LACTIC ACID ON *ENTODINIUM CAUDATUM* MONOCULTURE

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Abstract

This experiment was carried out to evaluate the effect of lactic acid on Entodinium caudatum monoculture in vitro. After thawing, E. caudatum was grown at 39°C under anaerobic condition to yield 10^5. Four groups were established by inclusion of 0, 0.5, 1, and 2 mM DL-lactic acid (Fluka Chemica, 69775). E. caudatum started to selectively use lactate to maintain 1.2 mM concentration at the highest lactic acid concentration. Increasing lactic acid concentration in medium was associated with reduction in pH (P<0.0001) and increase in total volatile fatty acids (P<0.0001), but no change in ammonia concentration. There was a reduction in acetate (P<0.04) and increases in propionate (P<0.02) and butyrate (P<0.0001) proportions as lactic acid concentration in medium increased. Stoichiometrically calculated gas production and CH4 amount increased accordingly with total volatile fatty acid production. In conclusion, E. caudatum grows to utilize lactate in case of acidosis.

Keywords: *Entodinium caudatum, lactic acid, in vitro rumen.*

INTRODUCTION

In order to meet energy demand, feeding excessive amount of readily fermentable carbohydrate sources can disturb rumen flora and microbial fermentation, which may result in acute and/or subacute acidosis (Umucalılar and Gülşen, 2005; Umucalılar et al., 2012).

Protozoa, especially ciliatas play a significant role in lactic acid metabolism in rumen when excess grains are fed. They engulf starch and soluble carbohydrates, which limits their utilization by amylolytic bacteria. This reduces lactic acid production (Nagaraja et al., 1992). Moreover, protozoa increases lactate fermentation, which reduces lactic acid accumulation in rumen (Nagaraja et al., 1992; Russell and Hespell, 1981). Entodiniomorphid ciliates help maintain ruminal pH (Dehority, 2005), by storing starch to minimize its utilization by starch-utilizing bacteria (Schwartzkopf-Genswein et al., 2003). This in vitro experiment was conducted to evaluate *E. caudatum* cultures in response to increased lactic acid concentration in medium.

MATERIALS AND METHODS

After thawing frozen *E. caudatum* cultures at 39ºC, they were allowed to grow in Medium M at 39ºC under anaerobic conditions to enumerate 10^5 (Dehority, 1998). Media were enriched 1.5% wheat flour and 1% ground alfalfa daily.

Cultures were then added with 0, 0.5, 1, and 2 m*M DL*-lactic acid (Fluka Chemica, 69775). Upon condensation, 1 of 10^{th} of the sediment were added with 96.6 ml Medium M, to achieve 10^3-10^4/ml. After incubation at 39ºC, 0.2 ml medium and 1.2 ml substrate solution were refreshed everyday at the same time. Every 3 d, half of the media was added with fresh Medium M (Dehority 1998). Media pH were measured before adding and 5 h after adding the substrate solution. On d 5 and refreshment of the media 1 ml sample was taken for determination of lactic acid and volatile fatty acid (VFA) concentrations and enumeration of protozoon.

Stoichiometrical Calculations (Blümmel et al., 1999):

CO_2 production (CO_2fer), mmol = acetate/2 + propionate/4 + 1.5 x butyrate

CH_4 production (CH_4fer), mmol = acetate + 2 x butyrate - CO_2
CO_2 released from buffer (CO_2buff), mmol = total VFA
Gas production, ml = (CO_2fer + CH_4fer + CO_2Isobutyrate + CO_2buff) x 0.0821 x 312
Methane level (Wolin, 1960; Ramin and Huntanen, 2012) was calculated using formula as follows:
Methane, ml = 22.4 x [(0.5 x acetate) – 0.25 x propionate) - (0.5 x butyrate) – (0.25 x valerate)]
In a completely randomized design experiment data were analyzed using 2-way ANOVA (SPSS, 2006).

RESULTS AND DISCUSSIONS

Increasing lactic acid addition up to 2 m*M* decreased pH (Table 1; Figure 1). Excess lactate appeared to be used by *Entodinium caudatum* to maintain its level by 1.2 m*M* (Figure 2).

Table 1. Effects of lactic acid addition on medium pH, ammonia, lactate concentrations and *Entodinium caudatum* numbers

Trt[1]	pH	Ammonia m*M*	Lactate m*M*	Protozoon
0	6.60	2.98	---	$4.7x10^3$
0.5	6.61	2.91	0.83	$6.6x10^3$
1	6.56	3.02	1.17	$8.1x10^3$
2	6.50	3.04	1.27	$9.4x10^3$
Effect	---------- *P* > F ----------			
Trt	0.0001	0.31	0.0001	0.0001
T	0.0001	0.0001	0.0001	0.0001
Trt x T	0.001	0.08	0.0001	0.0001

[1]Trt = treatments, lactic acid, mM. T = time, day.

Table 2 Effects of lactic acid addition to medium containing *Entodinium caudatum* monocultures on VFA profile and fermentation parameters

	Lactic Acid (m*M*)					
Parameters	0	0.5	1.0	2	SEM	*P* > F
Acetate (%)	56.4	57.8	54.9	52.8	0.84	0.040
Propionate (%)	22.2	21.5	23.6	24.3	0.44	0.017
Isobutyrate (%)	6.0	5.5	5.4	5.0	0.13	0.009
Butyrate (%)	9.1	9.1	10.1	12.5	0.32	0.000
Isovalerate (%)	4.9	4.8	4.5	4.0	0.27	0.572
Valerate (%)	1.4	1.2	1.5	1.4	0.07	0.373
Σ VFA (m*M*)	0.42	0.46	0.45	0.52	0.01	0.001
CO_2fer (ml)	0.20	0.22	0.22	0.27	0.01	0.000
CH_4fer (ml)	0.11	0.13	0.12	0.13	0.001	0.028
CO_2buff (ml)	0.40	0.43	0.42	0.49	0.01	0.000
Gas (ml)	18.8	20.4	20.0	23.6	0.45	0.000
NGR[1]	3.60	3.82	3.36	3.36	0.09	0.070
e-CH_4[2] (ml)	2.52	2.80	2.61	2.97	0.06	0.027

[1]NGR = nonglucogenic VFA:glucogenic VFA.
[2]e-CH_4 = estimated methane production.

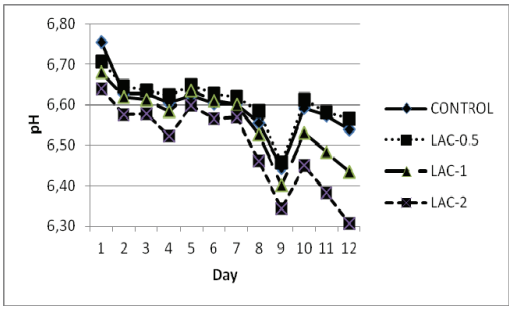

Figure 1. Alterations in pH in media containing *E. caudatum* upon addition of different concentrations of lactic acid (SEM = 0.06).

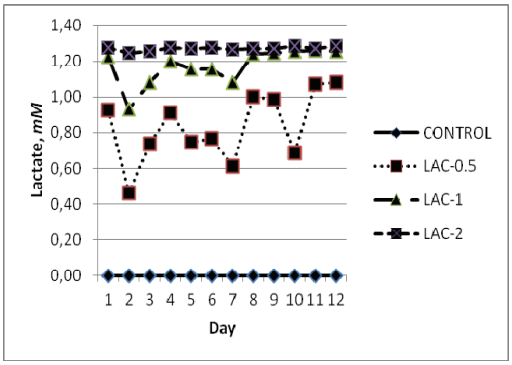

Figure 2. Alterations in lactate concentrations in media containing *E. caudatum* upon addition of different concentrations of lactic acid (SEM = 0.01).

While total VFA increased, proportion of acetate decreased and proportions of propionate and butyrate increased as concentration of lactic acid increased in media containing *Entodinium caudatum* (Table 2).

Increased lactic acid concentration caused increases in total VFA production and CO_2 as well as CH_4. Increased CO_2 release from buffer is a way to neutralize pH. These increases led to increases in stoichiometrically calculated gas production and CH_4 (Table 2).

CONCLUSIONS

Lactic acid inclusion up to 2 m*M* decreased pH in media containing *E. caudatum*. Reduction in pH associated with stimulation of *E. caudatum* to maintain pH, through modifying rumen fermentation.

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