IN VITRO ACTIVITY OF DIFFERENT ANTIMICROBIALS ON AMINO ACID DEAMINATION IN RUMINAL FLUID FROM DAIRY COWS FED CORN SILAGE OR CORN SILAGE/CONCENTRATE DIETS*

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INTRODUCTION

For many decades, antimicrobials have been used in livestock diets as growth-promoting agents (Berchielli, 2011). Monensin, an ionophore antibiotic frequently fed to cattle herds, has shown activity against several ruminal gram-positive bacteria and usually inhibits amino-acid deamination *in vitro* (Lima et al., 2009). Virginiamycin, an antibiotic produced by *Streptomyces virginae*, is generally effective against lactic acid bacteria and helps to maintain ruminal pH *in vivo* in conditions of induced acidosis (Coe et al., 1999). Although these antibiotics show desirable effects on ruminal fermentation, the growing concern with public health and the impact of antibiotics fed to livestock in selecting resistant bacteria has attracted attention to the development of alternatives to antimicrobials used for growth promotion (Russell & Mantovani, 2002). In this scenario, antimicrobial peptides (bacteriocins), such as nisin and bovicin HC5, have shown effects on ruminal fermentation that are similar to the ionophore antibiotics. In this study, we compared the *in vitro* effects of these two bacteriocins on amino acid deamination with the activity of monensin and virginiamycin. Because we hypothesized that inhibitory activity could be affected by microbiota composition determined by the diet, two dairy cows fed corn silage or high concentrate-based diets were used as rumen-fluid donors in this study.

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MATERIAL AND METHODS

Ruminal fluid samples were collected from two Holstein cows fistulated in the rumen that were fed corn silage (Diet A) or corn/sorghum silage and concentrate containing 22% CP (Diet B). The animals were adapted to the diets for 14 days before sampling. Rumen fluid was incubated at 39 °C for 48 h. The experiment was conducted in a completely randomized design with four treatments and three doses per treatment: bovicin HC5 (0, 180, and 360 AU/mL), nisin (0, 180, and 360 AU/mL), monensin (0, 1, and 5 µmol/L) and virginiamycin (0, 5, and 10 µmol/L). All incubations were performed in triplicate. Ammonia was determined by the colorimetric assay of Chaney and Marbach (1962). Absorbance was measured by spectrophotometry at 630 nm and ammonium chloride (NH_4Cl) was used as standard. Total ammonia (mmol/L) was expressed as the difference in ammonia concentration determined after 24 h of incubation and the initial concentration of ammonia (0 h). Data were subjected to analysis of variance and means were compared by the Tukey test at 5% probability using the Sisvar software.

RESULTS AND DISCUSSION

The addition of bacteriocins (nisin and bovicin HC5) to ruminal fluid of dairy cows fed corn silage (Table 1, Diet A) increased ($P<0.05$) ammonia concentration *in vitro*, while both monensin and virginiamycin decreased amino acid deamination under these experimental conditions. Ammonia concentrations increased by approximately 43% at a dose of 360 AU/mL of bovicin HC5 in diet A. These results differ from previous observations that indicated a reduction in ammonia production *in vitro* when an additional source of amino acids and peptides was added to the ruminal fluid (Lima et al., 2009). Virginiamycin and monensin reduced by approximately 21% the concentration of ammonia in a low-protein diet (diet A), but only at high doses (5 mmol/L and 10 mmol/L, respectively). Monensin was the only additive decreasing ammonia concentrations regardless of animal diet. The diet containing concentrate (22% CP) showed higher ammonia concentrations compared with the corn silage diet, and most inhibitors had no effect on amino acid deamination. Only monensin (5 µmol/L) decreased ammonia concentration in the concentrate-based diet. These results suggest that

conditions *in vitro* mimicking more closely the rumen environment (e.g. no dilutions of ruminal fluid in anaerobic media or addition of external substrates) could be useful to identify inhibitors that are potentially effective *in vivo*.

Table 1 - Ammonia concentration (mmol/L) in the presence of different inhibitors. Rumen fluid was obtained from cows fed a corn silage diet (Diet A) or a corn/sorghum silage and concentrate diet (Diet B). Ammonia concentration was determined after 24 h of incubation at 39 ºC

Inhibitor	Ammonia (Diet A)	P-value	Ammonia (Diet B)	P-value
Bovicin HC5 (AU/mL)		<0.01		0.39
0	8.55^{c}		38.46	
180	10.74^{b}		41.22	
360	12.26^{a}		38.64	
Nisin (AU/mL)		<0.01		0.79
0	12.81^{b}		24.02	
180	12.65^{b}		24.80	
360	15.64^{a}		23.30	
Monensin (µmol/L)		<0.01		0.03
0	14.59^{a}		25.43^{a}	
1	14.57^{a}		19.69^{ab}	
5	10.95^{b}		20.76^{b}	
Virginiamycin (µmol/L)		<0.01		0.97
0	15.48^{a}		22.87	
5	13.47^{a}		22.41	
10	12.09^{b}		22.73	
P-value (Bacteriocins		<0.01		<0.01

Means followed by different letters in the same column differ at 5% probability by Tukey's test. Statistical analysis was performed separately for each antimicrobial evaluated.

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REFERENCES

BERCHIELLI, T. T., PIRES, A. V. AND OLIVEIRA, S. G., 2011. *Nutrição de ruminantes.* 2 ed. Jaboticabal : Funep 616p. il.

CHANEY, A.L. AND MARBACH, E. P, 1962. Modified reagents for determination of urea and ammonia. *Clin Chem*, 8, 130-132.

COE, M. L., NAGARAJA, T. G., WALLACE, N., TOWNE, E. G., KEMP, K. E. AND HUTCHENSON, J. P., 1999. Effect of virginiamycin on ruminal fermentation in cattle during adaptation to a high concentrate diet and during an induced acidosis. *Journal of Animal Science* 77, 2259-2268.

LIMA, J.R., RIBON, A.O.B., RUSSELL, J.B. AND MANTOVANI, H.C., 2009. Bovicin HC5 inhibits wasteful amino acid degradation by mixed ruminal bacteria in vitro. *FEMS Microbiology Letters* 292, 78-84.

RUSSELL J. B. AND MANTOVANI H. C, 2002. The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. *J. Mol. Microbiol. Biotechnol.* 4, 347–355.