



## Influência de diferentes antimicrobianos na digestibilidade *in vitro* de dietas ricas em forragem ou concentrado<sup>1</sup>

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**Resumo:** Agentes antimicrobianos têm sido utilizados na nutrição de ruminantes para aumentar a eficiência alimentar e o desempenho animal. Os agentes antimicrobianos podem auxiliar na estabilização do pH ruminal, na redução das perdas de nitrogênio dietético e na redução da relação acetato/propionato. No entanto, a adição de antibióticos na alimentação de ruminantes não deve inibir a degradação de fibra. O objetivo deste estudo foi avaliar o efeito de bacteriocinas (bovicina HC5, nisina) e antibióticos (virginiamicina, monensina) sobre a digestibilidade *in vitro* da fibra em detergente neutro (DIVFDN) em duas dietas diferentes. Nisina e monensina não afetaram a DIVFDN ( $P > 0,05$ ), quando adicionadas a dieta A (silagem de milho) ou à dieta B (silagem de milho/sorgo e concentrado). Bovicina HC5 só diminuiu a DIVFDN ( $P < 0,05$ ) na dieta A quando utilizada na maior concentração testada (360 AU/mL) e a adição de virginiamicina à dieta A reduziu a DIVFDN ( $P < 0,05$ ), independentemente da concentração. Esses resultados demonstram que os agentes antimicrobianos testados neste estudo têm pouco efeito sobre a DIVFDN de dietas ricas em concentrado.

**Palavras-chave:** Bacteriocinas, ionóforos, ruminantes, silagem, fibra em detergente neutro

### Influence of different antimicrobials on the *in vitro* digestibility of forage-rich and concentrate-rich diets

**Abstract:** Antimicrobial agents have been used in livestock diets to increase feed efficiency and animal performance. Antimicrobial agents can help stabilize ruminal pH, reduce dietary nitrogen losses and acetate to propionate ratio. However, the addition of antibiotics in ruminant diets should not inhibit fiber degradation. The aim of this study was to evaluate the effect of bacteriocins (bovicin HC5, nisin) and antibiotics (virginiamycin, monensin) on *in vitro* digestibility of neutral detergent fiber (IVDNDNF) in two different diets. Nisin and monensin did not affect IVDNDNF ( $P > 0.05$ ) when added to diet A (corn silage) or diet B (corn silage/sorghum and concentrate). Bovicin HC5 only decreased IVDNDNF ( $P < 0.05$ ) in diet A at the highest concentration tested (360 AU/mL) and the addition of virginiamycin to diet A reduced IVDNDNF ( $P < 0.05$ ), regardless of the concentration tested. These results demonstrate that the antimicrobials tested in this study have little effect on IVDNDNF of concentrate-rich diets.

**Keywords:** Bacteriocins, ionophores, ruminants, silage, neutral detergent fiber

### Introduction

Antimicrobial agents are useful to improve the efficiency of nitrogen utilization and the overall ruminal fermentation in dairy cows. However, the effect of antimicrobials on fiber digestibility in forage-based or concentrate-based diets is not always clear. Although some studies have investigated the effect of ionophores on *in vitro* organic matter digestibility, most results were obtained using monensin as the tested antimicrobial.

It has been shown that monensin can improve feed efficiency by targeting populations of gram-positive ruminal bacteria. This effect is often associated with an increase in true protein escape from the rumen and greater nitrogen retention by the host animal. Similarly, studies have shown that virginiamycin may help stabilize ruminal fermentation and decrease variations in feed intake. *In vitro*, virginiamycin is a potent inhibitor of lactic acid bacteria and potentially useful in preventing rumen acidosis. Nonetheless, using antibiotics as feed additives have raised welfare and public health concerns due to the rise of antibiotic resistant bacteria among animals and humans in intensive livestock production systems. In this scenario, antimicrobial peptides produced by bacteria (bacteriocins) have been proposed as an alternative to modulate microbial activity in the rumen.

Callaway et al. (1997) demonstrated that nisin, a peptide produced by *Lactococcus lactis*, could increase propionate production and reduce methane production *in vitro*. Although nisin is widely used as a food



preservative, its application to manipulate ruminal fermentation is limited by the instability of the peptide in the rumen ecosystem. On the other hand, ruminal bacteriocins appear to be more stable in rumen fluid and could have greater effect on ruminal fermentation. Bovicin HC5, a bacteriocin produced by *Streptococcus bovis*, could inhibit methane production and amino acid deamination *in vitro* by mixed ruminal bacteria, suggesting potential benefits on ruminal fermentation (Lee et al., 2002; Lima et al., 2009). However, little work has been done to evaluate the impact of these antimicrobials on fiber digestibility *in vitro* and *in vivo*. Therefore, in this study we aimed to evaluate the effect of bacteriocins (bovicin HC5, nisin) and antibiotics (virginiamycin, monensin) on the *in vitro* digestibility of neutral detergent fiber (IVDADF) in forage-rich and concentrate-rich diets.

#### Material e Methods

Ruminal fluid samples were collected from two Holstein cows fistulated in the rumen fed corn silage (Diet A - Animal 1) or silage corn/sorghum and concentrate (Diet B - Animal 2). The animals were adapted to the diets for 14 days before sampling. Rumen fluid was incubated at 39°C for 48 hours. The experiment was conducted in a completely randomized design with four treatments and three doses per treatment: bovicin HC5 (0, 180 e 360 AU/mL), nisin (0, 180 e 360 AU/mL), monensin (0, 1 e 5 µmol/L) and virginiamycin (0, 5 e 10 µmol/L). All incubations were performed in triplicate. Samples from each diet were dried at 60 ± 5°C in an oven with forced ventilation and ground in a Willey mill, sieved through a mesh of 1 mm. The IVDADF was determined by the method proposed by Tilley and Terry (1963) with some modifications. 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 increasing doses of nisin and monensin to rumen fluid did not affect ( $P>0.05$ ) the *in vitro* digestibility of neutral detergent fiber (IVDADF) of corn silage with 8.7% crude protein (dry matter basis). However, the addition of bovicin HC5 (360 AU/mL) or virginiamycin (5 and 10 µmol/L) decreased ( $P<0.05$ ) the IVDADF in 6%, 13% and 14%, respectively (Table 1). When the diet consisted of silage corn/sorghum and concentrate (15.2% CP on a dry matter basis) no decrease ( $P>0.05$ ) in IVDADF was observed for any of the antimicrobial agents tested in this study, although the addition of 5 µmol/L virginiamycin increased ( $P<0.05$ ) the IVDADF in 10% compared to its higher dose (10 µmol/L).

Table 1. *In vitro* digestibility of neutral detergent fiber of diets A and B containing different doses of bovicin HC5, nisin, monensin and virginiamycin.

IVDADF	Diet A	P-Value	Diet B	P-Value
<b>Bovicin HC5 (AU/mL)</b>		<0.01		0.61
0	60.54 <sup>a</sup>		65.71	
180	62.04 <sup>a</sup>		65.63	
360	56.73 <sup>b</sup>		65.71	
<b>Nisin (AU/mL)</b>		0.79		0.80
0	60.72		63.47	
180	60.97		64.09	
360	61.27		62.51	
<b>Monensin (µmol/L)</b>		0.18		0.89
0	58.15		64.49	
1	57.50		63.35	
5	58.15		64.09	
<b>Virginiamycin (µmol/L)</b>		<0.01		0.04
0	63.37 <sup>a</sup>		61.78 <sup>ab</sup>	
5	55.86 <sup>b</sup>		65.96 <sup>a</sup>	
10	55.32 <sup>b</sup>		59.59 <sup>b</sup>	
<b>P-Value (Bacteriocins vs antibiotics)</b>		<0.01		0.30

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

Although results are controversial for the effects of monensin on *in vitro* fiber digestibility, our results are in agreement with previous observations performed with mixed forage and concentrate rations (Weimer et al., 2011).



Our results also agree with Coe et al.(1999) whom noted differential effects of virginiamycin on high-concentrate and high-forage diets *in vivo*. The present study is the first report of the effects of nisin and bovicin HC5 on *in vitro* fiber digestibility.

The average *in vitro* fiber digestibility of the corn silage diet (Diet A) and the corn silage/sorghum and concentrated diet (Diet B) were 59.2% and 63.8%, respectively. The IVDNDF of diets with different nutritional compositions are expected to differ and concentrate-rich diets (Diet B) often show higher IVDNDF compared to forage-rich diets (Diet A).

### **Conclusions**

These results indicate that the antimicrobial agents tested in this study did not inhibit fiber degradation in concentrate-rich diets. Virginiamycin decreased IVDNDF in the forage-rich diet while bovicin HC5 had a negative impact on IVDNDF in this diet only at the highest concentration tested. In general, the IVDNDF of bacteriocin-treated cultures were equivalent or greater than antibiotic-treated cultures, suggesting that antimicrobial peptides could be useful alternatives to these antibiotics. Further studies aiming to characterize the effects of these antimicrobials on ruminal fermentation and ruminal microbial community composition are needed to elucidate the mechanism of action of these antimicrobials in the rumen.

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