liferation of *E. coli* in the ruminant gut requires further knowledge of the behaviour of this bacterium in this ecosystem.

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1. Rasmussen M, Cray WC, Casey TA, Whipp SC (1993) *FEMS Microbiol Lett* 114, 79-84
2. Tan Y, Riley MA, (1996) *Microbiol* 142, 2175-2180
3. Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G (1994) *J Bacteriol* 176, 2137-2147

Production and characteristics of bacteriocins of rumen-associated enterococci. A Lauková[1], M Mareková[2], T Dobránsky [3], S Czikková[1] P Javorsky[1] (*[1]Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia; [2]University of P.J. Šafárik, Faculty of Medicine, Department of Chemistry and Biochemistry, SNP 1, 04001 Košice, Slovakia; [3]Institute of Neuroimmunology, Slovak Academy of Sciences, Dúbravská cesta 9, 80000 Bratislava, Slovakia)*

Enterococci represent the group of bacteria which form the obligate microflora of traditional cheeses in most Mediterranean countries. These species are also a part of the normal flora of the gastrointestinal tract and faeces of humans, various animals and poultry. In ruminants, enterococci are among the first bacterial groups colonizing the rumen wall as well as rumen fluid. On the other hand, the presence of enterococci in milk and milk products can be considered as an indication of inadequate sanitation during the production and processing of milk. They also often cause nosocomial gastrointestinal or urinary infections. Enterococci thus are an important group of microorganisms. Several strains are producers of bacteriocins [1]. In general, bacteriocins can be characterized as a heterogeneous group of proteins of varying molecular mass and biochemical properties with bactericidal effect against strains and species usually closely related to the producer culture. Bacteriocin producers have been isolated from food, silage or human sources. Newer bacteriocins from ruminal strains have been detected and characterized. Therefore, this study is relevant both to basic research and appropriate applications in veterinary medicine or the food industry.

Three small (3-10kDa) thermostable, hydrophobic bacteriocin-like substances, susceptible to proteases, were isolated. Two were produced by *Enterococcus faecium* strains CCM 4231 and EF3 of ruminal origin (calf and sheep). The third was produced by *E. casseliflavus* EC24, isolated from the rumen contents of deer. All proteineous substances showed a broad antimicrobial spectrum of activity (200 - 1600AU ml^{-1}). They inhibited the growth of Gram positive indicator bacteria. The best characterized was enterocin CCM 4231, which was also active against the G^- indicator organism *Proteus mirabilis*. The optimum pH, for enterocin CCM 4231 production was pH 4.0 - 7.5. Using controlled buffered systems (pH7) for cultivation of the producer strain, production of bacteriocin was constitutive. On the basis of their properties, these bacteriocins could be allotted to bacteriocin class II [2].

1. Olasupo NA, Schillinger CM, Franz AP, Holzapfel WH (1994) *Lett Appl Microbiol* 19, 438-441
2. Jack RW, Tagg JR, Ray B (1995) *Microbiol Rev* 59, 171-200

The effect of high lipid sheep diets on fatty acids of liquid and solid associated rumen bacteria. RJB Bessa, MA Almeida, JMR Ribeiro, AV Portugal (*INIA, Estação Zootécnica Nacional, Department of Nutrition, Fonte Boa, Vale de Santarém, 2000 Santarém, Portugal)*

Three rumen fistulated rams were used in a 3x3 Latin square experimental design in which the control diet (C) was dehydrated alfalfa pellets and wheat straw. The treatments consisted of soybean oil, mixed with milled alfafa before pelleting which resulted in final lipid contents of 8% (SO6) and 14% DM (S012). The rams were fed twice a day near maintenance (40g of lipid free DM $gLW^{0.75}$). The rumen contents were collected before the morning meal, and 1.5, 3 and 6 hours after that meal. The liquid and solid phases of the four collection times were pooled. The solid phase was washed with a 37°C isotonic solution and stored overnight at 4°C in a pH2 isotonic solution with 0.1% Tween 80 and treated as described by Whitehouse *et al* [1]. The samples were centrifuged for 20min at 500g and the resulting supernatants centrifuged for 30min at 20000*g*. Fatty acids (FA) analyses were made by a one-step extraction-methylation [2], using benzene as solvent and C19:0 as internal standard. Fatty acid methyl esters were analysed by GC.

The inclusion of soybean oil increased the FA contents of rumen bacteria although this effect was more evident in solid-associated (SAB) than liquid-associated bacteria (LAB). C18 acids contributed 80-90% of this increase. Among the C18 acids, C18:0 and C18:1 *trans*11 (C18:1*t*11) were the most important and contributed respectively 43 *vs* 31% in SO6 treatment and 28.5 *vs* 42% in SO12 treatment. This increase of C18:1*t*11 suggested a reduced ability for the complete hydrogenation of C18:2n-6. The quantity of odd-numbered FA (Odd-FA) did not vary between SAB and LAB

Fatty acid content of bacteria associated with the solid and liquid phases of rumen contents

Fatty acids	*C*		*SO6*		*SO12*				
	SAB	*LAB*	*SAB*	*LAB*	*SAB*	*LAB*	*SEM*	*T*	*P*
FA. Mg g^{-1}	156.0^{b}	67.2^{a}	310.8^{c}	123.3^{d}	350.9^{d}	121.0^{b}	11.19	*	*
C18:0	32.08^{bc}	19.37^{a}	37.87^{d}	29.86^{b}	33.37^{c}	20.46^{a}	0.99	*	*
C18:1*t*11	5.25^{a}	2.81^{a}	20.03^{bc}	14.01^{b}	23.84^{c}	21.80^{c}	2.28	*	
C18:1*cis*9	10.48^{d}	2.95^{a}	8.37^{c}	4.59^{b}	7.47^{c}	3.90^{ab}	0.43		*
C18:2n-6	1.86	1.62	1.98	1.78	2.13	1.78	0.40		
C18:3n-3	1.09^{b}	1.98^{c}	0.33^{a}	0.51^{a}	0.30^{a}	0.64^{ab}	0.14	*	*
Odd-FA	3.16^{b}	5.91^{c}	1.14^{a}	2.81^{b}	0.88^{a}	2.58^{b}	0.23	*	*
BC-FA	3.03^{b}	8.11^{d}	1.30^{a}	4.49^{c}	0.91^{a}	4.65^{c}	0.40	*	*

*Significance for error level P<0.01; T- treatment; P- SAB *vs* LAB. Averages with different index (a,b,c,d) are significantly different: SEM - standard error