IN VITRO ALTERATIONS IN RABBIT CAECAL METABOLITES BY ANTIMICROBIAL FEED ADDITIVES

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Abstract - We attempted to modify composition of microbial metabolites in the rabbit caecum by means of eight antimicrobial feed additives. Lincomycin and virginiamycin at 5 mg/l significantly ($P<0.05$) decreased production of methane, total VFA, molar percentage of acetate and decreased percentage of propionate in *in vitro* caecal cultures. Bacitracin increased proportion of butyrate and decreased that of caproate. Caproate was decreased also by lincomycin and tylosin. Avilamycin, avoparcin, nitrovin and spiramycin did not influence caecal fermentation. Fermentation shifts in treated cultures were generally small. It can be concluded that rabbit caecal fermentation is relatively insensitive to antimicrobial feed additives. Their beneficial effects on feed utilization are thus probably based on mode of action other than alterations of the fermentation stoichiometry in the caecum.

INTRODUCTION

Antimicrobial feed additives are often used to enhance the growth and feed efficiency or to treat digestive disorders in farm animals (Parker and Armstrong, 1987). It is generally believed that the performance - enhancing effect of antimicrobial feed additives can be attributed mostly to altered microbial activity in the gastrointestinal tract. In rabbits, the highest microbial activity exists in the caecum. The rabbit caecum is colonized by an abundant bacterial flora, which converts plant carbohydrates to volatile fatty acids (VFA), methane, carbon dioxide and compounds incorporated into bacterial cells. Volatile fatty acids are absorbed into the bloodstream and cover about 40% of the maintenance requirement (MARTY and VERNAY, 1984). There are studies which suggest that rabbit caecal metabolism resembles metabolism in the rumen (MAKKAR and SINGH, 1987; ADERIBIGDE and CHEEKE, 1993). In the rumen, antimicrobial feed additives increase propionate production and decrease methanogenesis and acetate to propionate molar ratio (DE JONG, 1989). The absorbed propionate is used mainly in glucose synthesis, thereby sparing other glucose precursors for synthetic functions. Acetate is oxidized to CO_2, leaving *ca* one-third for lipogenesis and other uses (BROCKMAN, 1993). Thus, it seems to be desirable to alter rabbit caecal fermentation pattern towards more propionate and less acetate. The aim of this study was to test eight antimicrobial feed additives in this respect.

MATERIAL AND METHODS

Mature, New Zealand White x Californian hybrids were used throughout the experiments. Rabbits were fed ad libitum a commercial granulated feed, containing (g/kg): dry matter - 908, crude protein - 155, fat - 19.4, crude fibre - 136, ash - 67.2. The feed contained no coccidiostat. Rabbits were killed, their caeca emptied and used for inoculation of *in vitro* cultures. Incubations were carried out in 0.5 l bottles. The caecal contents (50 ml) were diluted with 100 ml of a phosphate-bicarbonate buffer, containing urea and yeast extract (0.5 g N per litre) and sodium sulphide (0.5 g/l) as the reducing agent. Maize starch (1.0 g), wheat hemicellulose (0.5 g) and pectin (0.5 g) were added into each bottle. Antimicrobial feed additives were added at 0 or 5 mg/l, final concentration. Cultures were flushed with CO_2, hermetically closed witch rubber stoppers and incubated in quadriplicate on a water bath at 38°C for 8 h. The following antimicrobial feed additives were used: avilamycin, tylosin (Eli Lilly and Co., U.S.A.), avoparcin (Cyanamid, U.K.), bacitracin, nitrovin (Research Institute of Biopharmacy and Veterinary Drugs, Czech Rep.), lincomycin, spiramycin, (Fluka, Switzerland), virginiamycin (Smith Kline Beecham, Belgium).

Total VFA were estimated by titration, after steam distillation. Their molar composition was determined on a gas chromatograph equipped with a column of Supelcoport (Supelco, U.S.A.) and FI detector, at 140°C. Samples of the headspace gas were taken at the end of the incubation and analysed using the same

chromatograph. At the same time, the manometric pressure in incubation vessels was measured. Methane production was calculated as the product of methane concentration and total gas production. The results summarized in Table 1 are related to a difference between the beginning and the end of the incubation. The significance of differences was evaluated by the *t* - test.

Table 1 : Effect of antimicrobial feed additives on production of methane and volatile fatty acids in in vitro incubations of the rabbit caecal contents

Additive	Methane (mmol/l)	VFA (mmol/l)	C_2 %	C_3 %	C_4 %	C_5 %	C_6 %
Avilamycin	8.2	75.5	64.1	13.7	18.4	2.8	1.0
	(0.4)	(3.7)	(2.8)	(1.2)		(0.6)	(0.2)
Control	10.1	85.2	61.8	13.2	19.7	3.7	1.6
	(1.3)	(6.0)	(3.9)	(0.7)	(1.3)	(1.2)	(0.4)
Avoparcin	17.7	93.3	73.4	6.2	17.5	1.0	1.9
	(1.0)	(5.4)	(0.9)	(0.4)	(0.9)	(0.1)	(0.1)
Control	17.3	89.6	73.9	5.9	17.1	1.0	2.1
	(0.7)	(5.6)	(0.4)	(0.2)	(0.3)	(0.1)	(0.2)
Bacitracin	17.0	80.2	72.5	5.4	20.1*	0.9	1.1*
	(0.8)	(9.4)	(0.5)	(0.1)	(0.1)	(0.1)	(0.1)
Control	16.7	89.4	73.6	5.3	17.9	0.9	2.3
	(0.8)	(6.0)	(0.9)	(0.4)	(0.5)	(0.1)	(0.1)
Lincomycin	14.0*	60.6*	74.9*	6.2*	16.7	1.3	0.9*
	(0.8)	(2.0)	(0.3)	(0.1)	(0.2)	(0.2)	(0.1)
Control	17.3	89.6	73.9	5.9	17.1	1.0	2.1
	(0.7)	(5.6)	(0.4)	(0.1)	(0.3)	(0.1)	(0.2)
Nitrovin	14.5	88.1	69.2	9.9	16.4	1.8	2.7
	(1.7)	(6.6)	(1.2)	(0.8)	(0.5)	(0.1)	(0.8)
Control	14.5	85.4	70.5	9.3	15.4	1.8	3.0
	(0.9)	(6.2)	(0.9)	(0.5)	(1.4)	(0.1)	(0.4)
Spiramycin	15.5	76.9	73.5	6.4	16.7	1.2	2.2
	(1.3)	(5.7)	(0.4)	(0.2)	(0.3)	(0.1)	(0.1)
Control	15.5	77.6	72.8	6.5	17.5	1.2	2.0
	(1.3)	(8.0)	(1.0)	(0.6)	(0.6)	(0.1)	(0.1)
Tylosin	14.8	70.6	72.6	7.4	17.8	1.6	0.6*
	(0.5)	(5.3)	(0.4)	(0.2)	(0.4)	(0.2)	(0.1)
Control	15.4	73.3	73.8	6.8	16.8	1.3	1.3
	(1.4)	(6.3)	(1.0)	(0.6)	(0.6)	(0.2)	(0.2)
Virginiamycin	15.4*	72.8*	69.7*	11.1*	16.5	2.5	0.2
	(1.1)	(2.2)	(0.9)	(0.5)	(0.5)	(0.3)	(0.1)
Control	18.8	92.3	72.0	9.1	16.8	2.1	0.2
	(1.3)	(3.1)	(1.2)	(0.8)	(0.6)	(0.3)	(0.1)

Means of four replicates. Standard deviations are given in parentheses.
* Significantly different from the control ($P < 0.05$)

RESULTS AND DISCUSSION

Lincomycin and virginiamycin significantly decreased methane and total VFA production in cultures of the rabbit caecal contents (Table 1). Both additives significantly decreased molar percentage of acetate and increased that of propionate. Bacitracin increased molar proportion of butyrate ($P < 0.05$) and three additives (bacitracin, lincomycin, tylosin) significantly decreased production of caproate. Addition of other antimicrobials did not produce significant effects on metabolite formation.

Results of SZABO et al. (1988) and FEKETE et al. (1988) have shown that nitrovin and virginiamycin improve feed utilization in rabbits. Nitrovin is a synthetic antibacterial substance (a nitrofuran derivative). Other antimicrobials used in this study are feed antibiotics, active against grampositive bacteria in the alimentary tract. In the rumen, these compounds significantly alter fermentation pattern (RUSSELL and STROBEL, 1988; DE JONG, 1989). In the contrary, fermentation shifts observed in treated cultures of the rabbit caecal contents were relatively small in our study. Avilamycin, avoparcin, nitrovin and spiramycin did not produce any significant effect on formation of caecal metabolites. Tylosin influenced only caproate molar proportion. In our opinion, differences in action of antimicrobial feed additives in the rumen and rabbit caecum reflect different composition of microflora in both microbial ecosystems. Rumen microflora is more diverse as also protozoa and anaerobic fungi are present. It is possible that in the rabbit caecum, there are lower counts of grampositive bacteria and those bacteria which stain gramnegatively, but have a grampositive structure of the cell wall (butyrivibrios, ruminococci). Beneficial effects of antimicrobial feed additives in rabbits, thus, seem to be based on mode of action other than alterations of the fermentation stoichiometry in the rabbit caecum.

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REFERENCES

ADERIBIGDE A.O., CHEEKE P.R., 1993. Comparison of in vitro digestion of feed ingredients by rabbit cecal and bovine rumen fluids. *Anim. Feed Sci. Technol.* **41**, 329-339.

BROCKMAN R.P., 1993. Glucose and short-chain fatty acid metabolism. In: *Forbes, J.M. - France, J.: Quantitative Aspects of Ruminant Digestion and Metabolism.* C.A.B. International, Wallingford (U.K.), pp. 249-265.

DE JONG A., 1989. In vitro and in vivo alterations in ruminal volatile fatty acids by antimicrobial compounds. *AJAS*, **2**, 322-324.

FEKETE S., MAERTENS L., TLGYESI G., 1988. Digestion and faecal mineral content of virginiamycin-fed rabbits exposed to physiological and simulated stress. *Acta Vet. Hung.* **36**, 61-68.

MAKKAR H.P.S., SINGH B. 1987. Comparative enzymatic profiles of rabbit cecum and bovine rumen contents. *J. Appl. Rabbit Res.* **10**, 172-174.

MARTY J., VERNAY M., 1984. Absorption and metabolism of the volatile fatty acids in the hind-gut of the rabbit. *Brit. J. Nutr.* **51**, 265-277.

PARKER D.S., ARMSTRONG D.G., 1987. Antibiotic feed additives and livestock production. *Proc. Nutr. Soc.* **46**, 415-421.

RUSSELL J.B., STROBEL H.J., 1988. Effect of additives on in vitro ruminal fermentation : a comparison of monensin and bacitracin, another gram-positive antibiotic. *J. Anim. Sci.* **66**, 552-558.

SZABÓ S.L., HULLÁR I., GIPPERT T., 1988. Nitrovin in fattening of rabbits. *Proc. 4th Congress WRSA* (Budapest), pp. 173-177.