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FERMENTATION OF N-CONTAINING COMPOUNDS IN RABBIT CAECAL CULTURES

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Abstract - The fermentation pattern was investigated in anaerobic incubations of the rabbit caecal contents, diluted with buffer and supplied with plant proteins (gluten, gliadin, zein), mucin, casein, ribonucleic acid (RNA) and glucose. Parallelly, blank incubations were carried out by incubating caecal contents without substrate. Net production of volatile fatty acids (VFA) from casein and mucin was higher than from gluten, gliadin, zein and RNA. Zein was the least fermentable substrate tested. Acetate was the principal fermentation end-product, followed in most cultures by butyrate and propionate. Branched VFA accounted for 10.2 - 17.7 molar% in the total VFA produced from casein gluten, gliadin and zein. Fermentation of proteins produced more isovalerate than isobutyrate. In incubations supplied with proteins, there was a significant correlation between methane and VFA production, and between VFA and ammonia. Significantly more ammonia was found in cultures with casein than in other cultures. Production of methane was increased on glucose and nitrogenous substrates containing a carbohydrate moiety (mucin, RNA). Branched VFA proportions in VFA produced in cultures with glucose, mucin and RNA were lower than in those supplied with pure proteins.

INTRODUCTION

In rabbits, the caecum and proximal colon are important sites of organic matter digestion. Caecal microorganisms convert available nutrients (polysaccharides and nitrogenous substances) into mixture of metabolites (volatile fatty acids, methane, carbon dioxide, ammonia), and compounds of bacterial cells. The principal substrate of caecal bacteria are plant polysaccharides escaping the small intestinal digestion. It has been concluded that the characteristics of the substrate affect the fermentation pattern in carbohydrate-supplied cultures of caecal microorganisms (MAROUNEK *et al.,* 1997). No detailed information, however, is available on caecal fermentation of proteins and nucleic acids, in spite of the fact that the caecal contents are rich in compounds of nitrogen. HOOVER and HEITMANN (1975) reported the average value of 47 mg non-ammonia N per g of the dry matter of the caecal contents of rabbits fed a commercial concentrate feed. The presence of nitrogencus substances and ammonia levels in range 4 - 18 mmol/l (GIDENNE, 1997) suggest that the substantial decomposition of N-containing compounds in the rabbit caecum occurs. We suppose that the caecal fermentation of proteins and nucleic acids contribute to the total VFA production in the rabbit digestive tract. The aim of our experiments was to study fermentation pattern in cultures of the caecal contents supplied with plant proteins (gluten, gliadin, zein), mucin and nucleic acids. For comparison, fermentation pattern in cultures with casein and glucose was investigated.

MATERIALS AND METHODS

Three-month-old Hyla 2000 broilers were used. Rabbits were fed a granulated concentrate feed containing 30% alfalfa meal, 30% wheat bran, 12% barley, 8% wheat meal, 8% extracted soya-bean meal, 8% distillers dried grains, 1% rapeseed oil and 3% a vitamin-mineral supplement. The feed contained 17.0% crude protein, 13.5% crude fibre and 3.6% fat. Twelve rabbits were killed at *c.* 08.00 h., their caeca were emptied and pooled caecal contents were mixed and used for inoculation of *in vitro* cultures.

Incubations were carried out in quadriplicates on a shaking water bath at 39°C, in 100 ml bottles. The caecal contents (3 g) were added to 30 ml of the Burroughs buffer with 10% (v/v) of the caecal extract. The buffer was gassed with CO_2 before use. The caecal extract was prepared by autoclaving (110°C/45 min.) equal quantities of the caecal contents of rabbits and distilled water. The extract was clarified by centrifugation (8000 g/1 h), autoclaved and re-centrifuged before use. Substrates were added at 0.2 g per incubation. Casein, gliadin from wheat and mucin from hog stomach were purchased from Fluka. Gluten from wheat and zein from maize were purchased from Sigma. Sodium ribonucleate (RNA) from yeast was supplied by ICN. Lipids from gluten were removed by diethylether extraction. Sodium sulphide was added to the incubation fluid at 0.5 g/l as a reducing agent. Bottles were thoroughly flushed with CO_2, hermetically closed with rubber stoppers and incubated for 20 h. Blank incubations were carried out by incubating caecal contents without substrate.

Samples of the headspace gas were taken at the end of the incubation and analysed on a gas chromatograph with a flame-ionization detector. The chromatograph was equipped with a column of the Chromosorb WAW with 15% SP 1220 and 1% H_3PO_4 (Supelco). Total VFA were determined by titration, after steam distillation. Molar percentages of acetate (C_2), propionate (C_3), isobutyrate (i-C_4), butyrate (C_4), isovalerate (i-C_5) and valerate (C_5) were determined employing the gas chromatograph and the same column. Gas and VFA samples were analysed at the room temperature and 140 °C, respectively. Lactate was estimated by the microdiffusion method. Data were analysed statistically by one-way analysis of variance.

RESULTS

Productions of metabolites are presented in Table 1. The values were corrected for metabolites produced in corresponding incubated blanks. Incubation of proteins and RNA resulted in the net production of VFA, methane and ammonia, but not lactate. Proteins differed in their fermentability. Fermentation of casein and mucin yielded significantly more VFA than fermentation of other proteins (gluten, gliadin, zein) and RNA ($P < 0.001$). Zein was the poorest substrate out of six nitrogen compounds tested. In VFA produced from RNA significantly higher proportion of acetate and lower proportion of propionate was found ($P < 0.01$). Branched-chain VFA accounted on average for 10.2 - 17.7 molar% of the total VFA produced from casein, gluten, gliadin and zein. The quantity of isovalerate was greater than quantity of isobutyrate in all cultures, except those grown on glucose. Average production of methane and ammonia from proteins correlated significantly with that of VFA (r=0.96 and 0.94,

respectively; $P < 0.02$). Fermentation of casein produced significantly more ammonia than fermentation of other nitrogen-containing substrates ($P < 0.05$). Fermentation of nitrogenous substrates and glucose differed. Cultures of rabbit caecal microbes grown on glucose produced relatively more propionate and butyrate, and less branched-chain VFA than cultures supplied with proteins. Glucose and RNA fermentation yielded the same amount of methane, significantly more than fermentation of other substrates ($P < 0.01$). Ammonia was utilized instead of produced in glucose-supplied cultures.

DISCUSSION

Caecal microorganisms ferment crude protein composed of undegraded dietary protein passing from the ileum, protein of endogenous origin (enzymes and mucin secretions, cell desquamation) and nitrogen compounds released from lysed microbial cells. Presumably, undegraded dietary proteins are plant proteins of low solubility, i.e. prolamins and glutelins. Three proteins of this class have been included into our study (gluten, gliadin, zein). In contrast, casein is a phosphoprotein, well soluble in diluted alkali. As expected, insoluble proteins were fermented to a lesser extent than proteins soluble in the incubation fluid (casein, mucin). Mucin is a glycoprotein, present in saliva, gastric juice and intestinal juice. Carbohydrate moiety of nitrogen compounds influenced the fermentation pattern. Composition of fermentation end-products in cultures with mucin and RNA resembled to some extent end-products of glucose fermentation as (*i*) production of methane was significantly higher, and (*ii*) production of branched VFA lower in former cultures than in those supplied with pure proteins. Considerably higher acetate to propionate ratio in RNA-grown cultures may reflect difference between pentose (RNA) and hexose (glucose, mucin) fermentation. Purine and pyrimidine bases, i.e. products of nucleic acid decomposition are rather stable under anaerobic conditions, as shown in several rumen experiments (reviewed by SMITH, 1975), thus probably did not contribute to the VFA formation. Ammonia accumulation in our cultures, however, indicates that amino groups were deaminated at some stage of nucleic acid degradation. The fermentation of casein produced only 34% methane, compared with glucose. In ruminal experiments, casein produced only 50% of methane (DEMEYER and VAN NEVEL (1979) and 32% of gas (CONE and VAN GELDER, 1999), in comparison with carbohydrates. Fermentation of casein in our incubations yielded more VFA and less methane per g of substrate than fermentation of casein in the sheep rumen fluid, in experiment of DEMEYER and VAN NEVEL (1979). This can be related to differences between acetogenic (caecum) and methanogenic (rumen) type of fermentation, as suggested by DEMEYER *et al.,* (1989). Individual VFA differ in their physiological significance for the host. Acetate is the respiratory fuel and a precursor for lipogenesis and cholesterolgenesis. Propionate is gluconeogenic and a precursor of the synthesis of amino acids. Butyrate is the respiratory fuel of enterocytes. Branched VFA and valerate are growth factors of rumen cellulolytic bacteria (DEHORITY *et al.,* 1967). In ruminants, branched VFA have a general positive influence on rumen microbial metabolism, and are sometimes used as a feed additive to improve feed utilization (reviewed by ANDRIES *et al.,* 1987). Ammonia released from N-containing compounds is the main nitrogen source for growth of caecal microbes. The caecal fermentation of proteins and nucleic acids thus represents a supply of nutrients and growth factors which support optimal microbial growth, necessary for caecal organic matter digestion. In addition, caecal microbes

convert macromolecular nitrogenous compounds, which would be lost in hard faeces, into volatile products, available to the host.

Table 1. Total production (mmol/l) and molar percentages of volatile fatty acids (VFA), production of methane (mmol/l) and ammonia (mg/l) in *in vitro* incubations of rabbit caecal contents supplied with proteins, ribonucleic acid (RNA) and glucose. Standard errors are given in parentheses.

Substrate	Total VFA	C_2	C_3	i-C_4	C_4	i-C_5	C_5	CH_4	NH_3-N
Casein	42.9 (0.70)	41.9 (0.66)	15.5 (0.17)	6.6 (0.22)	16.3 (0.31)	11.1 (0.31)	8.6 (0.35)	1.17 (0.17)	557 (27.5)
Gluten	14.0 (0.95)	45.8 (1.98)	16.9 (0.68)	3.4 (0.43)	17.2 (1.00)	6.8 (0.81)	9.9 (0.95)	0.53 (0.11)	132 (12.5)
Gliadin	16.2 (1.28)	52.6 (1.92)	10.9 (0.73)	3.2 (0.56)	13.4 (0.85)	8.5 (0.75)	11.4 (1.15)	0.60 (0.27)	196 (19.2)
Zein	3.6 (0.79)	41.0 (1.29)	11.4 (0.98)	3.6 (0.80)	25.5 (1.53)	12.6 (1.15)	5.9 (0.89)	0.25 (0.08)	44 (10.9)
Mucin	44.7 (0.48)	60.4 (0.71)	16.9 (0.26)	1.7 (0.22)	14.8 (0.42)	2.2 (0.59)	4.0 (0.20)	1.71 (0.15)	387 (14.0)
RNA	21.2 (0.72)	78.7 (1.48)	4.7 (0.70)	-	12.7 (0.63)	1.8 (0.77)	2.1 (0.70)	2.85 (0.21)	424 (22.9)
Glucose	38.1 (1.07)	51.2 (0.75)	21.2 (0.27)	0.7 (0.13)	24.0 (0.54)	0.2 (0.21)	2.7 (0.23)	3.41 (0.11)	-80 (4.3)

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