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Microbial metabolism of plant 4-hydroxycinnamic and hydroxyalicylic acids in vitro. JH Pagella, XB Chen, WJ Shand, PS Dewey, ER Ørskov *(Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK)*

Benzoic acid (BA) is a common xenobiotic present in ruminant urine. It is the metabolic product of 3-phenylpropionic (PPA) and cyclohexanecarboxylic (CHCA) acids absorbed from the rumen. PPA is produced from cinnamic acid (CA) and from 4-hydroxycinnamic [*p*-coumaric (PCA), ferulic (FA), sinapic (SNA) and caffeic (CFA)] acids and CHCA from hydroxyalicylic [quinic (QA) and shikimic (SKA)] acids during the microbial fermentation of plant feeds. This study examined the production of PPA and CHCA from their precursors and the stability of PPA and CHCA using an in vitro incubation technique. Ten mg (45-78 μmol) of each of the substrates (PPA, CHCA, PCA, FA, CFA, SNA, CA, QA and SKA) was mixed with 90ml of incubation media in 100-ml conical flasks. The media consisted of 60ml of buffer solution and 30ml of rumen fluid from either rumen-defaunated or normal sheep. The flasks were flushed with CO_2 , sealed with a rubber stopper with a Bunsen valve and incubated at 39°C. All incubations were done in duplicate. Samples (4ml) of the incubation mixture were collected at 0.25, 2, 4, 8, 12, 24 and 48h after the start of incubation. The samples were analysed by gc.

Each of the PPA and CHCA precursors examined disappeared completely after 4h incubation. However, the recovery as PPA and CHCA were not complete. With the hydroxycinnamic acids, the recovery was higher when incubated with faunated than with defaunated rumen fluid, but with hydroxyalicylic acids, the recovery was higher with defaunated rumen fluid. SNA was not a precursor of PPA. The production of PPA from CA, which only involves a hydrogenation reaction, was rapid and reached a plateau at about 4h, indicating that hydrogenation would not be a limiting step causing the incomplete conversion of hydroxycinnamic acids to PPA. When PPA and CHCA were incu bated, their concentrations in the media remained unchanged over the 48h period, indicating that PPA and CHCA are not further metabolised by rumen microor ganisms. The results indicate that PCA,

Production of PPA and CHCA from their precursors after incubation with defaunated and faunated rumen fluid for 48h.

Precursor	*PCA*	*FA*	*CFA*	*SNA*	*CA*	*QA*	*SKA*
End product	PPA	PPA	PPA	PPA	PPA	CHCA	CHCA
Recovery (%)							
Defaunated rumen fluid	34(±6)	29(±2)	14(±2)	0(±0.2)	68(±0.3)	16(±0.3)	29(±0.01)
Faunated rumen fluid	67(±0.1)	64(±6)	54(±0.3)	0(±0.2)	96(±0.4)	6 (±0.5)	7(±0.6)

FA and CFA are the major contributors,and QA and SKA the minor contributors, of the BA excreted in ruminant urine. It appears also that protozoa differ from bacteria in their metabolism of hydroxycinnamic and hydroxyalicylic acids.

Applications of biomathematics to rumen microbiology. J France[1], J Dijkstra[2] ([1]*Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon EX20 2SB, UK;* [2]*Wageningen Institute of Animal Sciences, Department of Animal Nutrition, Wageningen Agricultural University, Marijkeweg 40, 6709 PG Wageningen, the Netherlands)*

The majority of mechanistic models appearing in the ruminant literature are based on systems of ordinary differential equations (ODEs). There is a mathematically standard way of representing such models called the rate:state formalism [1]. The system under investigation is defined at time t by q state variables which represent properties or attributes of the system, e.g. microbial mass, quantity of substrate. The model then comprises q first-order ODEs describing how the state variables change with time. The terms on the right-hand sides of these ODEs represent rates of component processes, e.g. microbial synthesis, substrate utilisation, and can be calculated from the values of the state variables and the parameters. There are three types of solution to these models. (i) The system under investigation is in steady state and solutions are obtained by setting the differentials to zero and manipulating to give an algebraic expression for each component process (Type I solution). (ii) The system is in non-steady state and the ODEs can be integrated analytically to give an expression for each state variable (Type II). (iii) The system is in non-steady state but the ODEs have to be integrated numerically (Type III). In this paper, we illustrate two applications drawn from our own work of mechanistic modelling in rumen microbiology based on the rate:state formalism with Type I and III solutions.

As an example of a Type I solution, an indirect approach to quantification of the fibrolytic anaerobic fungi in the rumen is described [2]. A model of the life cycle of anaerobic fungi, based upon observations of the life histories and growth kinetics of these organisms *in vitro* and *in vivo*, was constructed and solved in the steady state to determine the population of particle-attached fungal thalli from the concentration of free-swimming zoospores in rumen liquid. The values obtained are consistent with ruminal observations and with observations on faecal populations of fungal cysts or spores. As an example of a Type III solution, a model that simulates the dynamics of the rumen microbial ecosystem, with emphasis on the protozoa, is described [3, 4]. The model is driven by continuous inputs of nutrients and consists of 19 state variables representing the N, carbohydrate, fatty acid and microbial pools in the rumen. Several protozoal characteristics, e.g. engulfment and storage of starch, selective ruminal retention, are represented. The effects of dietary variations on microbial N turnover and recycling related to protozoal activity were simulated with diets containing roughages, e.g. fresh grass, maize silage, and variable proportions of concentrate, e.g. molasses, maize grain. High turnover rates and recycling levels were obtained with either maize silage, molasses or maize grain.