COMPARATIVE STUDY ON CAECAL FERMENTATION PATTERN IN ADULT DOMESTIC RABBITS AND WILD HARES

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ABSTRACT

Domestic rabbits and wild hares, despite their morphological resemblance and similar type of digestion, would differ in profile of caecal fermentation end-products. Without controlling the intake pattern of the two species, the caecal concentrations of total volatile fatty acids were higher and ammonia concentrations lower in rabbits than in hares (99 and 21 mmol/l vs. 47 and 33 mmol/l, resp.). Caecal microorganisms of rabbits produced more acetate (66 mmol/l) and butyrate (19 mmol/l) than propionate (10 mmol/l). Corresponding acetate, butyrate and propionate concentrations in hares were 28, 5and 9 mmol/l, resp. This was confirmed with *in vitro* experiment. In rabbit caecal cultures fermentation was accompanied with a significant methane release (15.3 ± 2.2 mmol/l). In hares only traces of methane were produced (0.1 mmol/l). Calculations of metabolic hydrogen recovery suggested that reductive acetogenesis exists in caeca of both animal species.

Key words: Rabbit, hare, caecum, fermentation, methane.

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*) are medium-sized herbivore animals with similar morphological features. They are both in the order *Lagomorpha*. In the natural environment the hare's diet is similar to the rabbit's diet. In both species the caecum is the primary site of digesta retention and microbial fermentation. Both rabbits and hares practise caecotrophy, i.e. produce two types of faeces, soft and hard, and ingest exclusively and totally the soft ones. Rabbits have been domesticated whereas hares are mainly wild animals, although some hare breeders exist in Europe. Comparative nutritional trials with rabbits and hares are difficult and thus scarce. Kuijper *et al*. (2004) carried out a feeding trial using rabbits and hares fed diets with a range of fibre contents. Dry matter digestibility was not different, but nitrogen digestibility was lower in hares than in rabbits, possibly because hares produced smaller amount of soft faeces. Both the stomach and the caecum were significantly smaller in the hare (as a proportion of body weight) than in rabbits: 2.5 ± 0.7% and 5.0 ± 1.5% in the hare, and 5.1 ± 1.4% and 6.8 ± 1.9% in rabbits, respectively (Stott, 2008). Both species moderately digest the cell-wall polysaccharides, but digestibility of hemicelluloses was significantly greater in the rabbit: 30 ± 5% in the hare and 39 ± 13% in the rabbit.

Caecal fermentation pattern in the domestic rabbits is well known, and depends of the delay from the last meal, and of the quantity and quality of the intake. Grossly, the caecal microorganisms of domestic rabbits produce VFA in the proportion of 60-80 moles of acetate, 8-20 moles of butyrate and 3-10 moles of propionate per 100 moles of VFA (Gidenne, 1996). Acetate is produced via glycolysis and by means of synthesis from CO_2 and H_2. Production of butyrate in rabbits exceeds that of propionate. Rabbits differ from almost all herbivorous animals, including ruminants which produce more propionate than butyrate in the rumen.

The present study has been aimed at extending our knowledge on digestive physiology of leporids. The concentrations of caecal metabolites were determined in the caecum of domestic rabbits and hares, as well as production of metabolites in cultures of caecal contents. Although hare breeding exist sporadically, the use of the same diet was not feasible in the present experiment, neither the control of the feed intake pattern.

MATERIALS AND METHODS

Domestic hybrid rabbits were fed *ad libitum* a commercial pelleted feed containing alfalfa meal, wheat bran, sunflower meal and oats as the main ingredients (Table 1). Eight rabbits were housed individually in cages and slaughtered at 9:00 a.m. at the age of 11 weeks. The caecal contents were squeezed out and used for (i) assay of caecal metabolites, and (ii) for inoculation of *in vitro* cultures. The caecal contents were immediately frozen or diluted 1:4 with phosphate-bicarbonate buffer (Burroughs *et al*., 1950). Caecal cultures were incubated in 320 ml bottles at 39°C for 8 h. The bottles were flushed with CO_2 and hermetically closed with rubber stoppers. The pH (about 7 initially) fell by ca 0.7 in the course of the incubation. Samples of the headspace gas were taken at the end of the incubation, then bottles were opened and the fermentation stopped by adding $HgCl_2$.

Table 1. Ingredients and calculated chemical composition of rabbit diet.

Ingredients (%)		Composition (g/kg)	
Alfalfa meal	28	Dry matter	907
Sunflower meal	19	Crude protein	169
Wheat bran	24	NDF	378
Sugar-beet pulp	4	ADF	224
Oats	13	ADL	56
Barley	7	Pectins	50
Rapeseed oil	2	Fructans	7
Vitamin supplement[a]	1	Starch	130
Dicalcium phosphate	0.5	Fat	45
Limestone	1	Digestible energy (MJ/kg)	10.2
Salt	0.5		

[a]Per kg supplement: vitamin A – 1 200 000 IU, vitamin D_3 – 200 000 IU, vitamin E – 5 g, vitamin K_3 – 0.2 g, vitamin B_1 – 0.3 g, vitamin B_2 – 0.7 g, vitamin B_6 – 0.4 g, niacinamide – 5 g, Ca - pantothenate – 2 g, folic acid – 0.17 g, biotin – 20 mg, vitamin B_{12} – 2 mg, choline – 60 g, lysine – 25 g, DL – methionine – 100 g.

The caecal contents of wild hares were obtained in November from eight animals (3.3 – 4.5 kg of weight) living in their natural environment near Osiek (Poland). The animals were trapped before noon using a soft net 400 m long, transported to Wroclaw and slaughtered in the afternoon. Samples of the caecal contents were taken for analyses and used for *in vitro* incubations as described above.

The headspace gas was analysed on a gas chromatograph equipped with a thermal conductivity detector. Total VFA were estimated by titration, after steam distillation. Their molar composition was determined on a gas chromatograph using a column of the Chromosorb WAW with 15% SP 1220/1% H_3PO_4 (Supelco). Ammonia was determined colourimetrically with Nessler reagent in Conway units. Metabolic hydrogen balance was calculated according to Demeyer (1991). Other analyses were performed as described previously (Marounek *et al*., 2005). The *t*- test was used to determine whether differences between rabbits and hares were statistically significant.

RESULTS AND DISCUSSION

Although the quantity and the quality (for the hares) feed intake of domestic rabbits and hares were not measured, neither the delay from the last meal, our results indicated that in domestic rabbits, caecal VFA concentration (98.9 mmol/l on average) was higher than average value of this parameter reported in healthy rabbits (Gidenne *et al.,* 2010) and approached the upper value of this trait reported by García *et al*. (2002). In wild hares, caecal VFA concentration, molar percentages of acetate and butyrate were lower and molar percentage of propionate higher than in rabbits ($P < 0.001$; see Table 2). Contrary to rabbits, caecal microorganisms of hares produced more other acids (valerate, caproate and isoacids) and ammonia. This finding has been confirmed in *in vitro* experiment (Table 3). High ammonia concentration and low VFA concentration suggest a shortage of fermentable substrate in the caecum of hares. A noteworthy exception, however, is the absence of methane production in hares:

only 0.09 – 1.6 ml/l headspace gas. Methane production represents an important hydrogen sink in ruminants and to a lesser extent also in adult rabbits. Alternative pathways for H_2 disposal in the digestive tract are reductive acetogenesis and reduction of sulphates. Metabolic hydrogen recovery in rabbit and hare caecal cultures was 50 and 55%, respectively. This suggests that reductive acetogenesis (synthesis of acetate from CO_2 and H_2), i.e. another hydrogen sink, exists in the caeca of both animal species. Molecular H_2 was not detected on GC records.

Table 2. Caecal metabolites in eight domestic rabbits and wild eight hares (mean values ± SD).

		Rabbits	Hares	P
Total VFA	(mmol/l)	98.9 ± 18.1	46.8 ± 14.0	< 0.001
Acetate	(mmol/l)	66.4 ± 3.3	28.4 ± 1.8	< 0.001
	(mol.%)	67.1 ± 3.3	58.7 ± 3.9	< 0.001
Propionate	(mmol/l)	10.1 ± 2.9	8.7 ± 1.0	< 0.001
	(mol.%)	10.2 ± 2.9	18.6 ± 2.1	< 0.001
Butyrate	(mmol/l)	19.7 ± 3.1	11.8 ± 4.0	< 0.001
	(mol.%)	19.7 ± 3.1	11.8 ± 4.0	< 0.001
Other VFA[a]	(mmol/l)	3.0 ± 1.5	5.1 ± 0.9	< 0.001
	(mol.%)	3.0 ± 1.5	10.9 ± 2.0	< 0.001
Ammonia	(mmol/l)	20.7 ± 8.0	33.4 ± 12.5	0.030

[a]Valerate, caproate and isoacids

The presence of methanogenic bacteria in fermentative parts of animal digestive tract primarily depends on the anaerobiosis and the availability of CO_2 and H_2 or formate. In ruminants methanogenesis is the main hydrogen sink, whereas in monogastric animals both methanogenesis and reductive acetogenesis occur together. Factors influencing the partitioning of H_2 between methanogenesis and acetogenesis are not fully understood. Methanogens seems to be sensitive to bile acids which may be present in the caecum but not in the rumen (Jezierny *et al.*, 2007). The study of Belenguer *et al.* (2011) has shown that methane formation estimated *in vivo* using a respiratory chamber was lower than methane production observed *in vitro*, probably due to the less favourable environmental pH (5.85 – 6.17 vs. 6.66 – 6.75). Furthermore, only some rabbits exhibited a remarkable methane production. Russell (1998) showed that rumen methane production was dramatically decreased at pH below 6.3. Hackstein and van Alen (1996) stressed that the presence of methanogenesis in various animal species was variable and not predictable.

Table 3. Production of volatile fatty acids and methane in cultures[a] of caecal contents of rabbits and hares (mean values ± SD).

		Rabbits	Hares	P
Total VFA	(mmol/l)	91.5 ± 9.7	113.9 ± 9.1	< 0.001
Acetate	(mmol/l)	69.8 ± 2.0	42.1 ± 3.2	< 0.001
	(mol.%)	76.3 ± 1.2	37.0 ± 2.8	< 0.001
Propionate	(mmol/l)	5.8 ± 0.7	34.4 ± 1.4	< 0.001
	(mol.%)	6.3 ± 0.3	30.2 ± 1.2	< 0.001
Butyrate	(mmol/l)	15.2 ± 1.3	17.4 ± 1.4	0.006
	(mol.%)	16.6 ± 1.4	15.3 ± 1.2	0.066
Other VFA[b]	(mmol/l)	0.7 ± 0.2	19.9 ± 1.5	< 0.001
	(mol.%)	0.8 ± 0.2	17.5 ± 1.3	< 0.001
Methane	(mmol/l)	15.3 ± 2.2	0.1 ± 0.1	< 0.001

[a]8 h – incubation; [b]valerate, caproate and isoacids

CONCLUSION

Incomplete metabolic hydrogen recoveries suggested that the reductive acetogenesis exists in caeca of both domestic rabbits and wild hares. In other fermentation traits, however, both animal species seemed to differ, although we should determine the effect of the quantity and quality of the intake for the two species. Caecal microorganisms of domestic rabbits produced more butyrate than propionate, whereas in hares more propionate than butyrate would be produced. In domestic rabbits the *in vitro* caecal fermentation was accompanied with a significant methane release. In hares only traces of methane were formed.

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