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УДК 577.12:579.22:636:92:639.112

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

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Due to the activity of microorganisms in the caecum of rabbits, acetate is formed in the result of processes of glycolysis and synthesis of CO_2 and H_2. In rabbits, there is peculiarity of fermentation of caecal microorganisms in greater butyrate production compared with propionate. The butyrate overproduction is characteristic only for rabbits in contrast to all herbivorous animals (including ruminants, in which, in the rumen, it's produced more propionate than butyrate). The research presented in this paper was aimed to increase knowledge about digestion in leporids. Therefore, it was defined the concentration of metabolites in fermentation processes in the caecum of rabbits and hares, and production of metabolites in cultures caecum content. It's important to note that the use of the same diet was not feasible in the present experiment because of sporadic rabbit breedings.

Eight hares (3.3–4.5 kg of weight) lived in their natural environment. In November, before noon, animals were trapped with a soft net (length, 400 m) and slaughtered in the afternoon. Eight rabbits were housed individually in cages and slaughtered at 9:00 a.m. at the age of 11 weeks. Samples of caecum content of rabbits and hares were analyzed, including measurement of concentrations of volatile fatty acids (VFA) and ammonium, and used for the cultivation of

microorganisms with subsequent determination production of VFA and methane.

Rabbits and hares, despite their morphological resemblance and similar type of digestion, differ in profile of caecal fermentation end-products. Caecal concentration of total volatile fatty acids were higher and ammonia concentrations was lower in rabbits than in hares (98.9 ± 18.1 and 20.7 ± 8.0 mmol/l vs 46.8 ± 14.0 and 33.4 ± 12.5 mmol/l, respectively). Caecal microorganisms of rabbits produced more acetate (66.4 ± 3.3 mmol/l) and butyrate (19.5 ± 3.1 mmol/l) than propionate (10.1 ± 2.9 mmol/l). Corresponding acetate, butyrate and propionate concentrations in hares were 28.4 ± 1.8, 5.5 ± 1.9 and 8.7 ± 1.0 mmol/l, respectively. This finding was confirmed in 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 suggest that reductive acetogenesis (an alternative electron sink) exists in caeca of both animal species. Thus, in rabbits caecal fermentation in vitro is accompanied by significant release of methane, while in hares it is produced in very small quantities.

Key words: RABBIT, HARE, CAECUM, FERMENTATION, AMMONIUM, METHANE.

ПОРІВНЯЛЬНЕ ВИВЧЕННЯ ЦЕКАЛЬНОГО ФЕРМЕНТАЦІЙНОГО СПЕКТРУ У ДОРОСЛИХ ДОМАШНІХ КРОЛІВ І ДИКИХ ЗАЙЦІВ

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Завдяки життєдіяльності мікроорганізмів-симбіонтів у сліпій кишці кролів ацетат утворюється внаслідок процесів гліколізу і синтезу з CO_2 і H_2. Головна особливість ферментації цекальних мікроорганізмів у кролів полягає в більшій продукції ними бутирату порівняно з пропіонатом. Така надпродукція бутирату властива тільки кролям, на відміну від усіх травоїдних тварин, включаючи жуйних, у рубці яких продукується більше пропіонату, ніж бутирату. Дослідження, представлені в цій роботі, були спрямовані на розширення знань щодо травлення в зайцеподібних. Тому, визначалися як концентрації метаболітів ферментаційних процесів у сліпій кишці кролів і зайців, так і продукція метаболітів у культурах вмістимого сліпої кишки. Слід відмітити, що утримування кролів на одній дієті було неможливим у цьому експерименті через їх спорадичне розведення.

Вісім зайців (масою 3,3–4,5 кг), які жили в природному середовищі, в листопаді у першій половині дня були відловлені за допомогою м'якої сітки довжиною 400 м, а в другій половині дня — забиті. Вісім кролів утримували індивідуально в клітках і у віці 11 тижнів забивали о 9 ранку. Досліджували зразки вмістимого сліпої кишки кролів і зайців. Зокрема, в них визначали концентрації летких жирних кислот (ЛЖК) і амонію, а також використовували для культивації мікроорганізмів з подальшим визначенням продукції ЛЖК і метану.

Встановлено, що кролі та зайці, незважаючи на їх морфологічну схожість і подібний тип травлення, відрізняються спектром кінцевих продуктів бродіння у сліпій кишці. Концентрація загального вмісту летких жирних кислот були вищою, а аміаку — нижчою у сліпій кишці кролів, ніж у зайців (98,9 ± 18,1 і 20,7 ± 8,0 ммоль/л проти 46,8 ± 14,0 і 33,4 ± 12,5 ммоль/л, відповідно). У сліпій кишці кролів мікроорганізми продукують більше ацетату (66,4 ± 3,3 ммоль/л) і бутирату (19,5 ± 3,1 ммоль/л), ніж пропіонату (10,1 ± 2,9 ммоль/л). Відповідні ж концентрації ацетату, бутирату і пропіонату в зайців

становили 28,4 ± 1,8, 5,5 ± 1,9 і 8,7±1,0 ммоль/л. Ці результати підтверджувалися в експериментах in vitro. У сліпій кишці кроля культуральна ферментація супроводжувалась значним викидом метану (15,3 ± 2,2 ммоль/л), а в зайців виявлено тільки слідові кількості метану (0,1 ммоль/л). За розрахунками метаболічного відновлення водню можна припустити, що відновний ацетогенез існує в сліпій кишці обох видів тварин.

Отже, у кролів цекальна ферментація in vitro супроводжується значним вивільненням метану, в той час, як у зайців він продукується в дуже незначній кількості.

Ключові слова: КРОЛИК, ЗАЄЦЬ, СЛІПА КИШКА, ФЕРМЕНТАЦІЯ, АМОНІЙ, МЕТАН

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ЦЕКАЛЬНОГО ФЕРМЕНТАЦИОННОГО СПЕКТРА У ВЗРОСЛЫХ ДОМАШНИХ КРОЛИКОВ И ДИКИХ ЗАЙЦЕВ

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Благодаря жизнедеятельности микроорганизмов-симбионтов в слепой кишке кроликов ацетат образуется вследствие процессов гликолиза и синтеза из CO_2 и H_2. Главная особенность ферментации цекальних микроорганизмов у кроликов состоит в большей продукции ими бутирата сравнительно с пропионатом. Такая сверхпродукция бутирата свойственная только кроликам, в отличие от всех травоядных животных, включая жвачных, в рубце которых продуцируется больше пропионата, чем бутирата. Исследования, представленные в этой работе, были направлены на расширение знаний относительно пищеварения в зайцеподобных. Поэтому определялись как концентрации метаболитов ферментационных процессов в слепой кишке кроликов и зайцев, так и продукция метаболитов в культурах содержимого слепой кишки. Следует отметить, что содержание кроликов на одной диете было невозможным в этом эксперименте из-за их спорадического разведения.

Восемь зайцев (весом 3,3–4,5 кг), которые находились в естественной среде, в ноябре в первой половине дня были выловлены с помощью мягкой сети длинной 400 м, а в другой половине дня — убиты. Восемь кроликов содержались индивидуально в клетках и в возрасте 11 недель были убиты в 9 часов утра. Исследовали образцы содержимого слепой кишки кроликов и зайцев. В частности, в них определяли концентрации летучих жирных кислот (ЛЖК) и аммиака, а также использовали для культивации микроорганизмов с последующим определением продукции ЛЖК и метана.

Установлено, что кролики и зайцы, несмотря на их морфологическое сходство и подобный тип пищеварения, отличаются спектром конечных продуктов брожения в слепой кишке. Концентрация общего содержания летучих жирных кислот была выше, а аммиака — ниже в слепой кишке кроликов, чем в зайцев (98,9 ± 18,1 и 20,7 ± 8,0 ммоль/л против 46,8 ± 14,0 и 33,4 ± 12,5 ммоль/л, соответственно). В слепой кишке кроликов микроорганизмы продуцируют больше ацетата (66,4 ± 3,3 ммоль / л) и бутирата (19,5 ± 3,1 ммоль/л), чем пропионата (10,1 ± 2,9 ммоль/л). Соответственные же концентрации ацетата, бутирата и пропионата в зайцев были 28,4 ± 1,8, 5,5 ± 1,9 и 8,7 ± 1,0 ммоль/л. Эти результаты

подтверждались в экспериментах in vitro. В слепой кишке кролика культуральная ферментация сопровождалась значительным выбросом метана (15,3 ± 2,2 ммоль/л), а в зайцев выявлены только следовые количества метана (0,1 ммоль/л). По подсчетам метаболического восстановления водорода можна предположить, что восстановительный ацетогенез существует в слепой кишке обеих видов животных.

Следовательно, у кроликов цекальная ферментация in vitro сопровождается значительным освобождением метана, тогда как в зайцев он продуцируется в очень незначительных количествах.

Ключевые слова: КРОЛИК, ЗАЯЦ, СЛЕПАЯ КИШКА, ФЕРМЕНТАЦИЯ, АММОНИЙ, МЕТАН

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 caecotrophagy, i.e. produce two types of faeces, soft and hard, and ingest only soft ones. Rabbits have been domesticated whereas hares are free-living animals, although some hare breeders exist in Europe. Comparative nutritional trials with rabbits and hares are scarce. Kuijper *et al*. [9] 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: 2.53 ± 0.72 % and 4.97 ± 1.49 %, respectively in the hare; 5.09 ± 1.38 % and 6.79 ± 1.87 % in rabbits, respectively [13]. Both species moderately digest the cell-wall polysaccharides, but digestibility of hemicellulose was significantly greater in the rabbit: 29.7 ± 4.5 % in the hare and 39.3 ± 12.5 % in the rabbit.

Caecal fermentation pattern in rabbits is well known. Caecal microorganisms of 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 [1, 6]. 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 rabbits and hares, as well as production of metabolites in cultures of caecal contents. Although rabbit breedings exist sporadically, the use of the same diet was not feasible in the present experiment.

Materials and methods

Rabbits were fed *ad libitum* a commercial pelleted feed containing alfalfa meal, wheat bran, sunflower meal and oats as the main ingredients (tabl. 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 [3]. 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 $HgCl_2$.

The caecal contents of 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.

All manipulations with rabbits and hares were carried out considering prescriptions of European Convention about protection of animals used in experimental and scientific purposes: euthanasia with intravenous injection of nembutal (18 % solution (200 mg/mL) drug dose of 200 mg/kg).

Table 1.

Ingredients and 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		

Note: [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 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 [4]. Other analyses were performed as described previously [11]. The *t*-test was used to determine whether differences between rabbits and hares were statistically significant.

Results and discussion

In rabbits, caecal VFA concentration (98.9 mmol/l on average) was higher than average value of this parameter reported in healthy rabbits [10] and approached the upper value of this trait reported by García *et al*. [5]. In hares, caecal VFA concentration, molar percentages of acetate and butyrate were lower and molar percentage of propionate was higher than in rabbits ($P < 0.001$; tabl. 2).

Table 2.

Caecal volatile fatty acids in eight rabbits and eight hares (mean values ± SD)

Volatile fatty acids		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

Note: [a]Valerate, caproate and isoacids

Contrary to rabbits, caecal microorganisms of hares produced more other acids (valerate, caproate and isoacids) and ammonia (fig. 1).

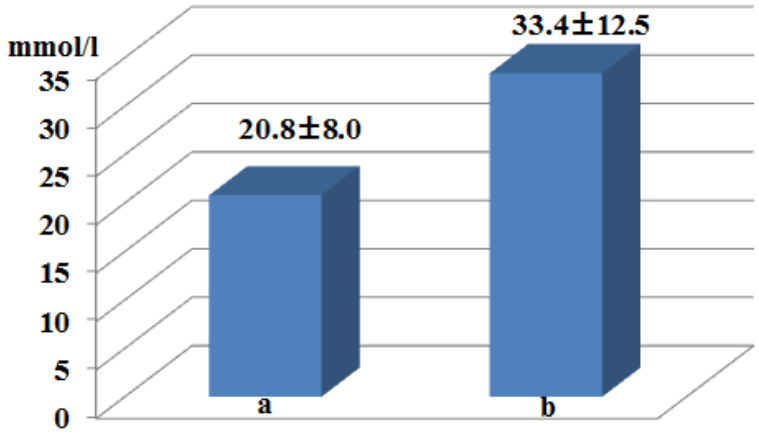

Fig. 1. Concentration of ammonia (mmol/l) in the ceacum of rabbit (a) and hare (b). (mean values ± SD; P=0.030)

This finding has been confirmed in *in vitro* experiment (tablю 3, fig. 2). 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 3.

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

Volatile fatty acids	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

Note: [a]8 h — incubation; [b]valerate, caproate and isoacids

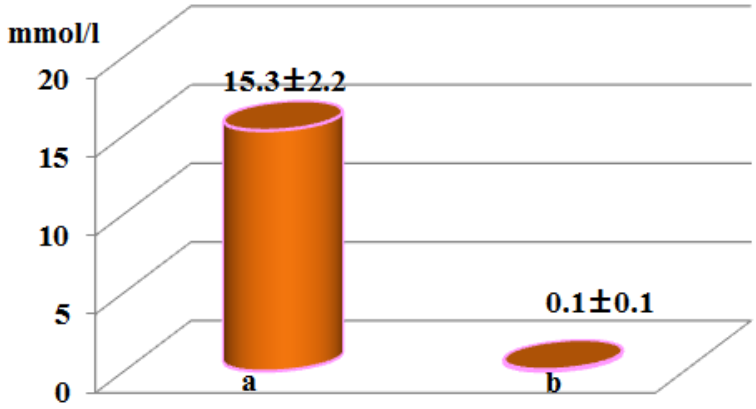

Fig. 2. Production of methane (mmol/l) by microorganisms in the ceacum of rabbit (a) and hare (b) during incubation (8 hours) *in vitro*. (mean values ± SD; P<0.001)

The presence of methanogenic bacteria in fermentive 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 [8]. The study of Belenguer *et al.* [2] 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 [12] showed that rumen methane production was dramatically decreased at pH below 6.3. Hackstein and van Alen [7] stressed that the presence of methanogenesis in various animal species was variable and not predictable.

Conclusion

Incomplete metabolic hydrogen recoveries suggest that the reductive acetogenesis exists in caeca of both rabbits and hares. In other fermentation traits, however, both animal species differ. Caecal microorganisms of rabbits produced more butyrate than propionate whereas in hares more propionate than butyrate was produced. In rabbits the *in vitro* caecal fermentation was accompanied with a significant methane release. In hares only traces of methane were formed.

Acknowledgements. This study was supported by institutional research projects AVOZ 5045 0515 and MZe 0002701404. Preliminary results were presented as a poster at World Rabbit Congress in Sharm-el-Sheikh (Egypt) 3.9.–6.9. 2012.

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