THE EFFECT OF RABBIT'S AGE ON *IN VITRO* FERMENTATION OF STARCH, COMPOUND FEED AND ITS FIBRE

DJELOVANJE STAROSTI KUNIĆA NA *IN VITRO* FERMENTACIJU ŠKROBA, SMJESE KRMIVA I NJEGOVE VLAKNINE

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SUMMARY

In vitro gas production movement for three different substrates, starch, standard compound feed (20 % crude protein, 33 % NDF/kg DM) and neutral detergent fibre prepared from the standard compound feed (NDF), were determined using the caecum content of weaned rabbits (36 days of age) and of rabbits of slaughter age (78 days) as inoculum. Gas produced was fitted with the Gompertz model and the differences between parameters were calculated. The differences in fermentation kinetic parameters between older and weaned rabbits were significant within each substrate. In rabbits at slaughter weight the fermentation was more intensive, more rapid and, with the exception of compound feed, the production of gas was higher than in weaned rabbit. In first 10 hours of fermentation that correspond to the normal retention time in the caecum (Gidenne *et al.*, 2000), the highest amount of gas was produced from compound feed. Only in this substrate the time of maximum fermentation rate was short enough (TMFR: 36 days 9.5, 78 days 6.6 hours, P<0.05) that it could be fermented *in vivo*. In accordance with published results it could be suggested that the majority of gas derived from pectins, which are important ingredients of compound feeds.

Key words: rabbit nutrition/ *in vitro* gas production/ fermentation/ starch/ compound feed/ NDF

INTRODUCTION

In rabbits, the caecum and proximal colon are important sites of digestion. Approximately 40 % of the digested organic matter of the feed is digested in the caeco-colic segment (Gidenne, 1992). Caecal microorganisms ferment available nutrients, mainly polysaccharides, which can not be digested by endogenous enzymes (hemicelluloses, pectins, cellulose) to short-chain fatty acids (SCFA), ammonia and gases (hydrogen, carbon dioxide and methane). Stable microbial fermentation is essential for the health of rabbit. Disturbances in nutrients availability can lead to microbial dysbiosis and irregular microbial fermentation, which result in digestive disturbances and increased mortality.

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The caecal digestive physiology differs between young and adult rabbits. Before weaning caecal microorganisms produced no methane and a considerable amount of ammonia; a major characteristics of caecal fermentation is reductive acetogenesis, which converts CO_2 and H_2 to acetate (Piattoni et al., 1996; Marounek et al., 1999). At the weaning the substrate for caecal fermentation change from milk to solid feed which contain large amounts of polysaccharides. It is not clear whether changes of fermentation pattern in the weaning period are caused by the change of substrate or reflect a sequential colonisation of the caecum by various bacterial groups.

The colibacilli flora count decreased linearly after day 15 of age and is stabilised on low level after day 42, while cellulose degrading microflora is completely absent before day 15 (before weaning) and increase and stabilise at day 36. Starch degrading microflora is high and stable from day 15 to day 49 (Padliha et al., 1995). Fibrolytic activity of the caecal microflora increase during weaning period: the pectinolytic and xylanolytic activities are high and at day 25 of age they reach 64 and 68 % of the activity observed at the day 44, respectively (Gidenne et al., 2002). The intake of milk may also influence the caecal fermentation as the rabbit milk fat contains antimicrobial compounds (Canas-Rodriguez and Smith, 1966).

The microbial fermentation of the caecum microorganisms can be estimated using in vitro gas production technique similar to that in ruminants (Piattoni et al., 1996; Marounek et al., 1997, 1999, 2000a, 2000b).

The objective of present work was to obtain and compare the in vitro gas production kinetic parameters of different substrates in the caecum content of weaned rabbits (36 days of age) and of rabbits of slaughter age (78 days).

MATERIAL AND METHODS

Substrates

Three substrates were used in the present study: starch (potato starch, Art. No. 101252, Merck, Darmstadt, Germany), standard compound feed for rabbits after weaning (20 % of crude protein (CP) and 33 % of NDF in dry matter (DM); Krka, Novo mesto, Slovenia) and the NDF prepared from this compound feed.

The NDF was prepared according to the modified method of Stefanon et al. (1996). The compound feed was weighed (1 g) into 250 ml baker and boiled in 100 ml of neutral detergent solution at 105 °C for 1 hour. The extracted fibre was filtered through 50 μm mesh nylon bag and washed several times with hot distilled water and then rinsed with ethanol and acetone. The bags were then dried at 45 °C until they were almost dry and were then incubated overnight at 25 °C with 1M ammonium sulphate to remove any trace of ionically bound detergent. The wash with ethanol and acetone was repeated and then the fibre was dried overnight at 45 °C.

In vitro fermentation

The inoculum was prepared according to the modified method of Calabro *et al.* (1999). Freshly collected samples of caecal content were used to prepare the inoculum. Two 78 days old or three 36 days old New Zealand White rabbits (Slovenian meat line SIKA) were randomly chosen prior to slaughtering. The animals were fed with a commercial compound feed described before as substrate. This diet was fed *ad libitum* from weaning at 35 days of age. The fed was withdrawn 12 hours before the sampling, but water was still available *ad libitum*. After slaughtering the caecum was isolated by tying off the two extremities with nylon string to prevent movement of the digesta.

The caecum contents were diluted (1:50 w/v) with the buffer solution prepared according to Menke and Steingass (1988), and then squeezed through 4 layers of cheese cloth to constitute the inoculum. During this procedure the microbial suspension was kept at 39 °C anaerobically under a stream of CO_2 gas.

In vitro gas production was determined according to the procedure described by Menke and Steingass (1988). One hundred and seventy five milligrams of sample were anaerobically incubated in triplicate in 100 ml glass syringes containing 30 ml of inoculum. The production of gas resulting from a microbial fermentation was measured manually at regular intervals at 0, 2, 4, 6,

8, 10, 12, 24, 36, 48, 72 and 96 hours to measure the incubation kinetics. Together with samples the standard hay and blank were also incubated in triplicate and blanks were used to obtain the total correct gas production.

Calculations and statistical analysis

Gas produced from the samples was initially corrected for the gas produced in blank samples and corrected data were fitted with the Gompertz model (Bidlack and Buxton, 1992; Lavrenčič *et al.*, 1997):

$$Y_t = B \times e^{-C \times e^{-At}}$$

where:

Y_t is the gas produced (ml/g DM) at time »t«, »B« is the asimptotic value of the gas production (ml/g DM), »C« is specific gas production rate, affected by a constant »A« describing the decay in specific gas production rate (Beuvink and Kogut, 1993), and »t« is time in hours. Parameter values and curve fitting were estimated by the marquard compromise of a non linear regression method, using the SAS software (Proc NLIN) (SAS, 1988).

The variation of gas production rates was obtained from the first derivative of Gompertz model with respect to time of incubation:

$$\frac{dY}{dt} = B \times C \times A \times e^{-At} \times e^{-C \times e^{-At}}$$

The time of maximum fermentation rate (TMFR; h) for gas production rate were calculated by setting the second derivative of the Gompertz model to equal 0 and solving for »t«:

$$\frac{d^2Y}{dt^2} = A \times B^2 \times C^2 \times (e^{-At})^2 \times e^{-C \times e^{-At}} - A \times B \times C^2 \times e^{-C \times e^{-At}}$$

Maximum fermentation rate (MFR; ml/h) for gas production was then calculated by using the respective value of TMFR in the first derivative equation.

The SAS software (Duncan subroutine of Proc GLM) (SAS, 1988) was used to calculate differences between the parameters obtained by incubating the substrates in the two inoculums deriving from rabbits of different ages.

RESULTS AND DISCUSSION

Table 1 and Figures 1 to 3 report the parameters from the Gompertz model. The potential gas production varied widely from 75.3 ml/g of NDF for 36 day old rabbits to 313.7 ml/g of starch at 78 days of age.

Table 1. Parameters of starch, compound feed and its NDF *in vitro* gas production with the inoculum from caecum content of 36 and 78 day old rabbits

Tablica 1. Parametri škroba, smjese krmiva i njezina NDF *in vitro* proizvodnja plina s inoculumom iz sadržaja caecuma kunića starih 36 i 78 dana

	Starch - Škrob		Compound feed - Smjesa krmiva		NDF	
	36[†]	78[†]	36	78	36	78
B (ml/g)	188.3[a]	313.7[b]	178.6[a]	142.8[b]	75.3[a]	92.3[b]
C	5.83[a]	8.446[b]	3.864[a]	4.139[b]	7.297[a]	6.320[b]
A	0.0643[a]	0.1208[b]	0.1425[a]	0.2163[b]	0.1222	0.1234
MFR (ml/h)	4.5[a]	13.9[b]	9.4[a]	11.6[b]	3.4[a]	4.2[b]
TMFR (h)	27.6[a]	17.7[b]	9.5[a]	6.6[b]	16.3[a]	14.9[b]

[†] = the age of rabbits (36 or 78 days) - starost kunića (36 i 78 dana)

[ab] = the values in rows within the same substrate with different superscripts are significantly different at P < 0.05

[ab] = vrijednosti u redovima unutar istog supstrata s različitim natpisima = značajno se razlikuju kod P < 0.05
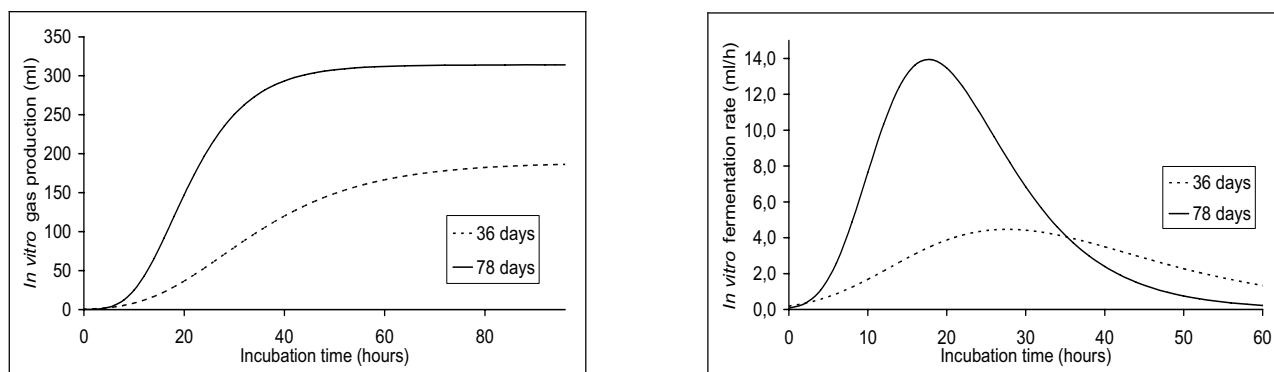
Figure 1. Starch accumulative fermentation curve and fermentation rates with the inoculum from caecum contents of 36 and 78 day old rabbits

Slika 1. Krivulja akumulativne fermentacije škroba i stopa fermentacije s inoculumom iz sadržaja caecuma kunića starih 36 i 78 dana
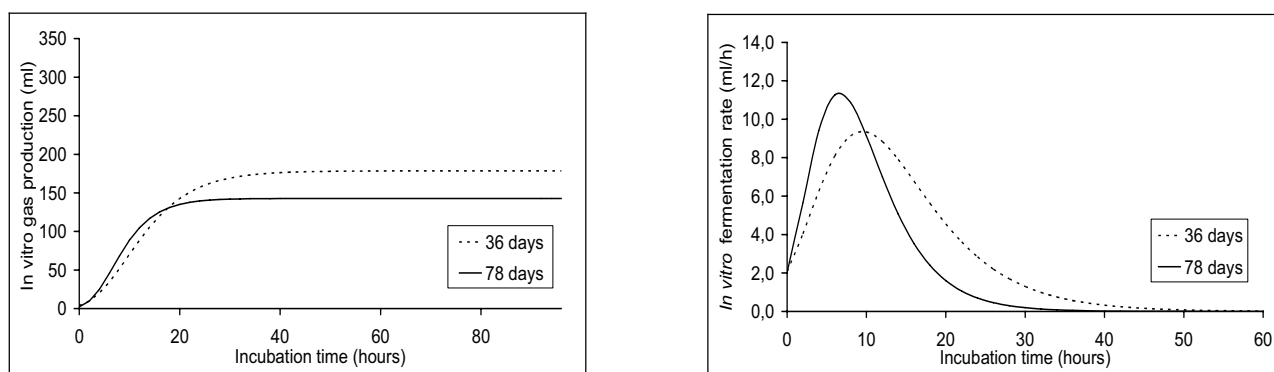

Figure 2. Compound feed accumulative fermentation curve and fermentation rates with the inoculum from caecum contents of 36 and 78 day old rabbits

Slika 2. Krivulja akumulativne fermentacije smjese krmiva i stope fermentacije s inoculumom iz sadržaja caecuma kunića starih 36 i 78 dana
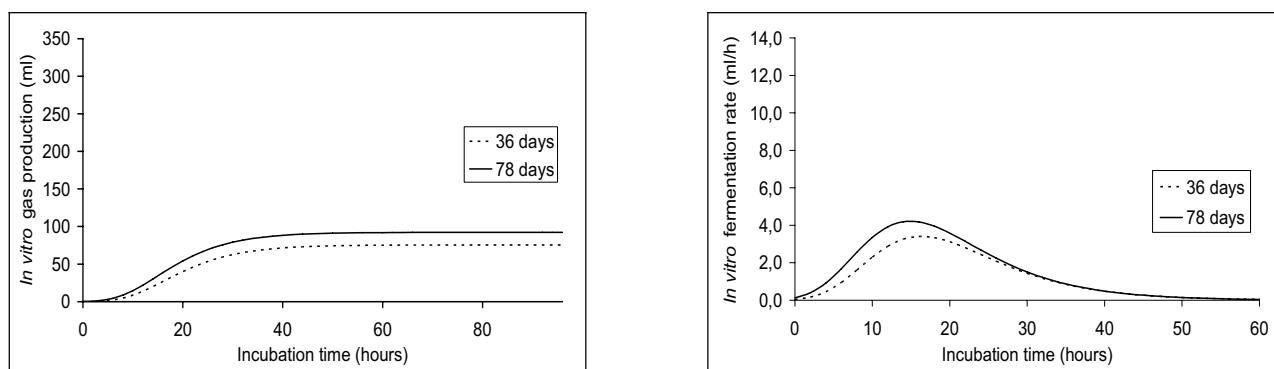

Figure 3. NDF accumulative fermentation curve and fermentation rates with the inoculum from caecum contents of 36 and 78 day old rabbits

Slika 3. Krivulja NDF akumulativne fermentacije i stope fermentacije s inoculumom iz sadržaja caecuma kunića starih 36 i 78 dana

In all tree substrates the differences between older and weaned rabbits were significant (P<0.05). In older rabbits the fermentation was more intensive (higher MFR), more rapid (shorter TMFR) and, with the exception of compound feed, the production of gas was higher than in weaned rabbits (higher cumulative gas production, parameter B of the equation).

The enzymatic system of the rabbit, weaned between 30 and 35 days of age, is not yet fully developed (Gidenne and Fortun-Lamothe, 2002), and some nutrients pass undigested through the small intestine into caecum, where microflora is established as soon as in the second week of age. The bacterial community increased steadily with the age and reached the stable levels around weaning (Bennegadi *et al.*, 2003). Padliha *et al.* (1995) found high levels of amylolytic microflora in caecum from day 15 to day 49 with a small decrease around weaning.

Marounek *et al.* (1999) found that the caecal fermentation was inhibited by rabbit milk which contains antimicrobial compounds (octanoic and decanoic acids) (Canas-Rodriguez and Smith, 1966). At these circumstances the reductive acetogenesis is a major characteristic of caecal fermentation in suckling rabbits (Piattoni *et al.*, 1996; Marounek *et al.*, 1999). Before weaning caecal microorganisms do not produce methane but considerable amounts of ammonia (Piattoni *et al.*, 1996; Marounek *et al.*, 1999). This probably can explain quite a slow gas production from starch and NDF in weaned rabbits observed in our experiment (Table 1 and Figures 1 and 3).

In our experiment the greatest differences between weaned and older rabbits were observed in the fermentation pattern of starch (Table 1 and Figure 1). Gas production with the inoculum of older rabbits was more intensive, rapid and the volume of gas produced was higher than with inoculum from weaned rabbits. Caecal microflora of older animals (at slaughter age) could be better adapted to starch, entering the caecum. But Gidenne *et al.* (2000, 2004) found very low ileal flow of starch (1.5 g/day), irrespective of age, ADF content of feed or content and quality of ingested starch. These authors concluded that starch did not affect significantly fermentative activity in caecum and emphasized that much higher importance is attributed to digestible fibre, mainly pectins.

Our results confirmed this finding. *In vitro* fermentation of starch was very low in the first 10 hours (Table 1 and Figure 1); this time corresponds to the caecum retention time of standard feed (Gidenne *et al.*, 2000). Furthermore, our results and high counts of amilolytic microorganisms in caecum (Padliha *et al.*, 1995) indicate, that caecal microflora is able to digest starch, but the retention time is so short that starch as substrate for caecum microorganisms does not have any importance.

The pattern of NDF fermentation was very similar to above mentioned starch fermentation. The TMFR (>10 h) and MFR (<5 ml/h) of NDF were very low (Table 1 and Figure 3) which is in accordance with the results of Gidenne *et al.* (2000, 2002) and Gidenne and Fortun-Lamothe (2002) who demonstrated that the caecal microflora exhibit high xylanolytic and low cellulolytic activities.

Only the fermentation of compound feed started without any delays (Table 1 and Figure 2). Compound feed contained a high amount of crude protein (20 %), starch, hemicelluloses and pectins (from cereals, dehydrated alfalfa and beet pulp, respectively). The contribution of crude protein and starch to gas production from compound feed is probably small, because Marounek *et al.* (2000b) found that the fermentation of plant proteins is low (only 30 % of glucose fermentation) and fermentation of starch is slow. We believe that the amount of gas produced from compound feed arises mainly from fermentation of pectins (and xylans). Gidenne *et al.* (2000, 2002) and Gidenne and Fortun-Lamothe (2002) found that the count of pectinolytic microorganisms and their activities were higher than counts and activities of xylanolytic or cellulolytic microbes. Marounek *et al.* (1997, 2000a) confirmed these observations. They observed that the *in vitro* fermentation of pectins was faster and the microorganisms produced more gas than with fermentation of hemicelluloses and xylans.

Marounek *et al.* (2000a) found higher production of metabolites from pectin degradation in 28 day old rabbits compared with 3 month old animals. Gidenne *et al.* (2002) and Gidenne and Fortun-Lamothe (2002) found that ability of caecal microflora to degrade cell wall (mainly pectins and xylans) is well established at weaning and do not change with age. This is in accordance with our

results, where gas production from compound feed in weaned rabbits was even higher than in older ones (Table 1 and Figure 2).

CONCLUSIONS

• The differences among older and weaned rabbits were significant in fermentation of all tree substrates. In rabbits at slaughter weight the fermentation was more intensive, more rapid and, with the exception of compound feed, the production of gas was higher than in weaned rabbits.

• In first 10 hours of fermentation that correspond to the normal retention time in the caecum (Gidenne *et al.*, 2000), the highest amount of gas was produced from compound feed. Only in this substrate the time of maximum fermentation rate was short enough (TMFR<10 hours) that it could be fermented *in vivo*. In accordance with published results it could be suggested that the majority of gas derived from pectins, which are important ingredients of compound feeds.

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SAŽETAK

Određena je in vitro kinetika proizvodnje plina za tri različita supstrata, škroba, standardne smjese krmiva (20% sirovih bjelančevina, 33% NDF/kg DM) i neutralnog deterdžentne vlaknine pripremljene iz standardne smjese krmiva (NDF) upotrebom sadržaja caecuma odbijenih kunića starih 36 dana i kunića u dobi za klanje (78 dana) kao inoculum. Proizveden plin spojen je s Gompertz kalupom/modelom pa su izračunate razlike između parametara. Razlike u kinetičkim parametrima fermentacije između starijih i odbijenih kunića bile su značajne unutar svakog supstrata. U kunića klaoničke težine fermentacija je bila intenzivnija, brža i uz iznimku smjese krmiva, proizvodnja plina bila je veća nego u odbijenih kunića. U prvih 10 sati fermentacije, što odgovara normalnom vremenu retencije u caecumu (Gidenne et al, 2000) najveća količina plina proizvedena je od smjese krmiva. Samo u ovom supstratu vrijeme maksimalne stope fermentacije bilo je dovoljno kratko (TMFR: 36 dana 9.5, 78 dana 6.6 sati, P<0.05/ da se moglo fermentirati in vivo. U skladu s objavljenim rezultatima može se pretpostaviti da većina plina potječe od pektina, važnih sastojaka smjese krmiva.

Ključne riječi: hranidba kunića, in vitro proizvodnja plina, fermentacija, škrob, smjesa krmiva, NDF