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THE *IN VITRO* CAECAL FERMENTATION OF DIFFERENT STARCH SOURCES IN RABBITS

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ABSTRACT

The objective of our study was to extend the knowledge of microbial fermentation of starch in the rabbit caecum using the *in vitro* gas production technique. Different sources of starch (wheat and maize grain, raw and cooked potato) and different starch isolates (wheat, maize and potato starch) were incubated with inoculum, prepared from rabbit caecum content. The gas production parameters such as total potential gas production (parameter "B"), gas production till 10 h of incubation (Gas10), maximum fermentation rate (MFR) and time of maximum fermentation rate (TMFR) were estimated with Gompertz model and were significantly affected by the substrata. Differences between maize grain and maize starch were small: MFR and TMFR were not different, while Gas10 of maize starch was higher than from maize grain (5 and 3 ml/kg DM, respectively; $P < 0.05$). MFR of wheat starch was higher than that of wheat grain (3.1 and 2.4 ml/h, respectively; $P < 0.05$), but reached maximum rate later (TMFR: 12.4 and 10.9 h, respectively; $P < 0.05$) and had lower Gas10 (6 and 9 ml/kg DM, respectively; $P < 0.05$). The fermentation of cooked potato reached the TMFR after 8.5 h of incubation and had the highest MFR (2.9 ml/h) and Gas10 (16 ml/kg DM) of all substrata. The MFRs of raw potato and potato starch were similar (2.1 and 2.3 ml/h, respectively), although TMFR and Gas10 of raw potato were shorter and higher than those of potato starch (TMFR of 10.7 and 17.2 h and Gas10 of 10 and 1 ml/kg DM, respectively; $P < 0.05$).

Key words: rabbits / animal nutrition / *in vitro* fermentation / gas test / caecum / starch

1 INTRODUCTION

Starch is important nutrient in rabbit feed (about 20% of the diet) and it is almost completely digested in the digestive tract of rabbits. Starch digestion takes place mainly in the small intestine with digestive enzymes. The efficiency of starch digestion can be affected by the age of rabbit, dietary level and origin of starch. With less effective digestion in small intestine the starch overload in the caecum can occur, which can provoke microbial dysbiosis and consequently digestive troubles and mortality (Gidenne and Garcia, 2006).

The stability of caecum microbial fermentation is essential for rabbit health. Nutrients which enter the caecum enable colonization of abundant microbial population which is able to ferment proteins, starch and fibre (Emaldi *et al.*, 1979; Makkar and Singh, 1987; Padilha *et al.*, 1995; Gidenne *et al.*, 2000, Gidenne *et al.,* 2002). Padilha *et al.* (1995) found high counts of amilolytic and low counts of cellulolytic microorganisms, what indicate, that starch can be important nutrient for caecal microorganisms.

Recently many authors found that starch digestion is very efficient even in young rabbits (5 weeks of age): the ileal digestibility of starch was over 97% and ileal flow of starch was very low (Gidenne *et al.*, 2000; Nicodemus *et al.*, 2004; Trocino *et al.*, 2011). Thus Gidenne and Garcia (2006) stated that starch overload in the caecum is very unlikely to occur, but more research about starch fermentation in rabbit caecum is needed.

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Table 1: Chemical composition of experimental substrata (g/kg DM)

Substrate	DM g/kg	Crude protein	Crude fat	Crude fibre	Crude ash	Nitrogen free extract
Maize grain	871	78.1	33.7	27. 9	12.5	847.8
Wheat grain	883	117.7	12.0	35.8	17.9	816.6
Potato tuber – raw	206	95.2	0.5	43.6	51.3	809.4
Potato tuber – cooked	208	98.5	2.2	34.8	49.6	814.8

The objective of our study was to extend the knowledge of microbial fermentation of starch in the caecum using the *in vitro gas* production technique. For this purpose different sources of starch (wheat and maize grain, raw and cooked potato) and different starch isolates (wheat, maize and potato starch) were incubated in inoculum, prepared from rabbit caecum content.

2 MATERIAL AND METHODS

2.1 SUBSTRATA

Four starch containing feeds, maize grain, wheat grain and potato tuber (raw and cooked) were used in the experiment. All substrata were ground (particle size 1 mm) prior to incubation; their chemical composition is given in Table 1.

In addition tree isolates were used: starch isolated from maize grain (Sigma S4126, 881 g/kg DM), starch isolated from wheat grain (Sigma S5127, 884 g/kg DM) and starch isolated from potato tuber (Sigma S4251, 865 g/kg DM).

Table 2: Parameters of the Gompertz model of different starch containing substrata

Substrate	B[†] (ml/g DM)	C[†]	A[†]
Isolated maize starch	37[a]	15.11[bc]	0.206[bcd]
Isolated wheat starch	35[a]	19.89[ab]	0.238[ab]
Isolated potato starch	37[a]	18.59[ab]	0.169[d]
Maize grain	32[b]	26.37[a]	0.233[ab]
Wheat grain	30[b]	10.47[cd]	0.215[abc]
Potato tuber – raw	32[b]	6.80[d]	0.178[cd]
Potato tuber – cooked	31[b]	8.74[cd]	0.253[a]

[†] B = asymptotic volume of the produced gas (total potential gas production), C = specific gas production rate, A = the decay in specific gas production rate;
[a, b, c, d] = means in columns with different superscripts are significantly different at $P < 0.05$

2.2 ***IN VITRO*** FERMENTATION

The caecum content of two 78 days old New Zealand White rabbits of Slovenian meat line SIKA were chosen to prepare the inoculum. Manipulations and selection of animals and the preparation of inoculum were performed according to the methods used by Lavrenčič (2007). *In vitro* gas production was determined according to the procedure described by Menke and Steingass (1988). One hundred and seventy five milligrams of air dry substrate were anaerobically incubated at 39 °C in duplicate in a 100 ml glass syringe containing 30 ml of inoculum.

2.3 CALCULATIONS AND STATISTICAL ANALYSIS

Measured *in vitro* gas production data were corrected for substrate dry matter content and for the gas produced from blank samples. Corrected values were then fitted to the Gompertz model (Lavrenčič *et al.*, 1997). Parameter values and curve fitting were estimated by the Marquard compromise of a non-linear regression method, using SAS software (Proc NLIN) (SAS, 2001). From the estimated parameters of Gompertz model other parameters were calculated: maximum fermentation rate (MFR; ml/h), the time of maximum fermentation rate (TMFR; h) and volume of gas produced until 10 hours of incubation (Gas10). Statistical analysis was performed by GLM procedure using the SAS software (SAS, 2001).

3 RESULTS AND DISCUSSION

The estimated parameters of *in vitro* gas production are reported in Table 2. There were significant differences in fermentation kinetic parameters according to the substrate.

The total potential gas production (parameter B of Gompertz model) were significantly higher ($P < 0.05$) when isolated starch were used in comparison with starch containing feeds. There were no differences be-

Table 3: *Maximum fermentation rate (MFR), time of maximum fermentation rate (TMFR) and gas produced till 10 hours of incubation (Gas10) from different starch containing substrata*

Substrata	MFR (ml/h)	TMFR (h)	GAS_{10} (ml/g DM)
Isolated maize starch	2.8^{ab}	13.2^{bc}	5^{c}
Isolated wheat starch	3.1^{a}	12.4^{c}	6^{c}
Isolated potato starch	2.3^{cd}	17.2^{a}	1^{d}
Maize grain	2.8^{abc}	14.0^{b}	3^{d}
Wheat grain	2.4^{bcd}	10.9^{d}	9^{b}
Potato tuber – raw	2.1^{d}	10.7^{d}	10^{b}
Potato tuber – cooked	2.9^{a}	8.5^{e}	16^{a}

$^{a, b, c, d}$ = means in columns with different superscripts are significantly different at the level P < 0.05

tween separate starch isolates as well as between separate starch containing feeds.

The parameter B is normally obtained only after prolonged incubation of substrata in the inocula (30 hours and more). In normal conditions in gastrointestinal tract the retention time in caecum is much shorter, only about 10 hours (Gidenne *et al*., 2000). Thus additional parameters such as maximum fermentation rate (MFR), time of maximum fermentation rate (TMFR) and gas produced until 10 h of incubation (Gas10) can describe better the fermentation pattern of the substrata (Table 3 and Fig. 1).

All these parameters were significantly ($P < 0.05$) affected by the substrate. Comparison between feed and starch isolated from the same feed shows that the differences in maize were small: MFR and TMFR of maize or isolated maize starch were not very different, but after the 10 hours of fermentation (Gas10) more gas was produced from isolated maize starch than from maize grain.

Fermentation of isolated wheat starch was higher (MFR) than that of wheat grain, but reached maximum rate later (eg. it had longer TMFR), so the amount of gas, produced after 10 hours of fermentation was lower (lower Gas10) from isolated wheat starch than from wheat grain. These differences can be caused by higher differences in nutrient contents between wheat grain and isolated wheat starch than between maize grain and isolated maize starch. It is obvious that wheat grain had higher crude protein content and consequently lower starch content than isolated wheat starch, while crude protein content in maize grain were much lower thus the starch contents being much more similar between isolated maize starch and maize grain.

The fermentation pattern of raw and cooked potato and isolated potato starch greatly differed. The fermentation of cooked potato reached the most rapidly the highest fermentation rate (eg. it had the shortest TMFR) and also fermented the most intensively (eg. it had the highest MFR and Gas10) of all substrata used. The MFRs of raw potato and isolated potato starch did not differed from each other, however the fermentation of raw potato reached the TMFR earlier and produced higher amount of gas after 10 hours of incubation than isolated potato starch. Gas10 of isolated potato starch was the lowest of all substrata used. We suppose that starch isolation procedure changed the properties of starch resulting in changed fermentation characteristics.

It is well known that starch is almost completely

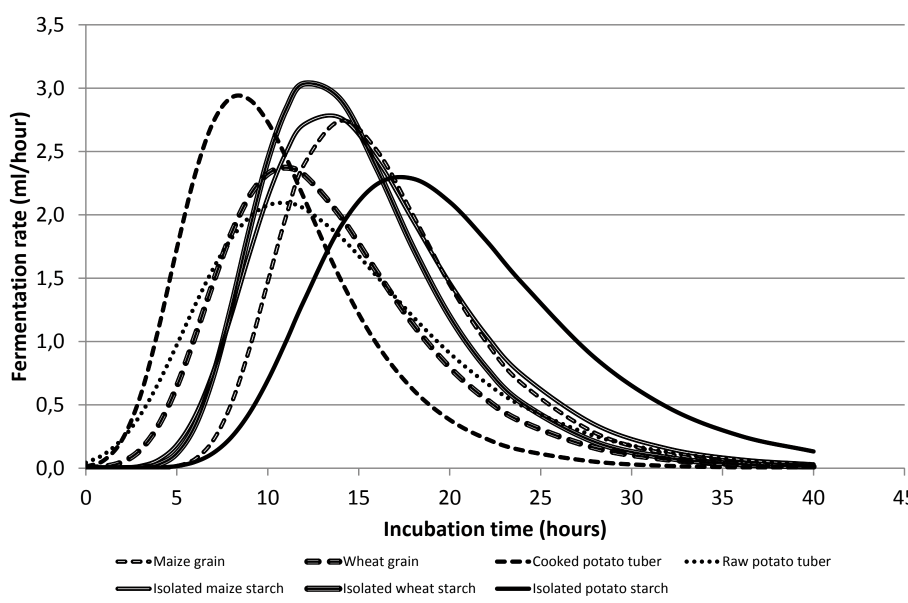

Figure 1: *Fermentation rate of substrata incubated in the inoculum prepared from the rabbit caecum content*

digested before it reaches the caecum, thus the scarce fermentation of starch in the caecum content of rabbits is not surprising. Gidenne *et al.* (2000) found that the ileal digestibility of starch (mainly from wheat) was 93 to 99% and ileal flow of starch was only 0.9 to 2.9% of DM. Even if the diets with high starch contents (22.6% DM) were fed to early-weaned rabbits (25 days of age) the ileal starch flow was not higher than 2.25 g/day (Gutiérrez *et al*., 2002a). On the other hand, Padilha *et al.* (1995) found high counts of amilolytic microbes in the rabbit caecum and the results of Lavrenčič (2007) indicated that some starch can reach the caecum because the *in vitro* fermentation of starch was higher in adult rabbits than in weaned.

The fermentation of all starch substrata in our experiment (except that of cooked potato) was low and slow, what is in accordance with the results of Lavrenčič (2007), Kermauner (2007) and Lavrenčič and Kermauner (2011) and confirmed findings, that starch is not important nutrient in rabbit caecum. Awati *et al.* (2005) found similar TMFRs (11.6 and 14.6 h) when they incubated wheat starch in the inoculum prepared from pig faeces.

The source of starch can have an influence on its fermentation in rabbit caecum as well. When wheat grain and soybean meal were replaced by peas the ileal flow of starch was higher (Gutiérrez *et al*., 2002b). Nizza and Moniello (2000) established that the ileal flow of starch from maize (1.9 to 2.2% of DM) was higher than from wheat (1.3 to 1.8% of DM), while Blas *et al.* (1994) found much higher ileal flow (3 to 7% DM) of maize starch. These findings indicate that caecal microorganisms can be better adapted to maize starch and higher fermentation can be expected when maize grain is used as source of starch in diets. However, our results do not support these results because the MFR of maize grain was obtained later (eg. maize grain reached later the TMFR) and had the Gas10 lower than wheat grain, while the MFRs between maize and wheat grain did not differ. Lanzas *et al.* (2007) found *in vitro* gas production of wheat and barley grain in the inocula prepared from rumen fluid started sooner than fermentation of maize and sorghum as well. When we used starch isolates, there were no differences between the calculated fermentation parameters of wheat and maize starch.

4 CONCLUSIONS

In the first 10 h of fermentation that correspond to the normal retention time in the caecum (Gidenne *et al*., 2000) the highest amount of gas was produced from cooked potato. However, only with this substrate the time of maximum fermentation rate was short enough (TMFR less than 10 h) that it could be extensively fermented also *in vivo*. The fermentation characteristics of all other starch containing feeds and isolates were so slow or low that we can assume that the starch from these substrata do not contribute significantly to the fermentation in rabbit caecum *in vivo*.

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6 REFERENCES

Awati A., Konstantinov S.R., Williams B., Akkermans A.D.L., Bosch M.W., Smidt H., Verstegen M.W.A. 2005. Effect of substrate adaptation on the microbial fermentation and microbial composition of faecal microbiota of weaning piglets studied in vitro.Journal of the Science of Food and Agriculture, 85: 1765–1772

Blas E., Cervera C., Fernandes Carmona J. 1994. Effect of two diets with varied starch and fibre levels on the performances of 4–7 weeks old rabbits. World Rabbit Science, 2: 117–121

Emaldi O., Crociani F., Matteuzzi D. 1979. A note on the total viable count and selective enumeration of anaerobic bacteria in the caecal content, soft and hard faeces of rabbit. Journal of Applied Bacteriology, 46, 1:169–172

Gidenne T., García J. 2006. Nutritional strategies improving the digestive health of the weaned rabbits. In: Recent advances in rabbit sciences. Maertens L., Coudert P. (eds.). Melle, ILVO: 229–238 http://world-rabbit-science.com/Documents/Cost848.pdf

Gidenne T., Pinheiro V., Falcao-E-Cunha L. 2000. A comprehensive approach of the rabbit digestion: consequences of a reduction in dietary fibre supply. Livestock Production Science, 64: 225–237

Gidenne T., Jehl N., Segura M., Michalet-Doreau B. 2002. Microbial activity in the caecum of the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. Animal Feed Science and Technology, 99: 107–118

Gutiérrez I., Espinosa A., García J., Carabaño R., De Blas J.C. 2002a: Effect of levels of starch, fiber and lactose on digestion and growth performance of early-weaned rabbits. Journal of Animal Science, 80: 1029–1037

Gutiérrez I., Espinosa A., García J., Carabaño R., De Blas J.C. 2002b: Effect of starch and protein sources, heat processing and exogenous enzymes in starter diets for early-weaned rabbits. Animal Feed Science and Technology, 98: 175–186

Kermauner A. 2007. Comparison of in vitro fermentation of starch, xylan, pectin, cellulose and compound feed in inocula prepared from caecotrophs and from the caecum content of rabbits. In: 15. Arbetstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle, 9–10 May 2007. Giesen, DVG: 79–87

Lanzas C., Fox D.G., Pell A.N. 2007. Digestion kinetics of dried cereal grains. Animal Feed Science and Technology, 136: 265–280

Lavrenčič A. 2007. The effect of rabbit age on in vitro caecal fermentation of starch, pectin, xylan, cellulose, compound feed and its fibre. Animal, 1: 241–248

Lavrenčič A., Kermauner A. 2011. The relationship between in vitro short-chain fatty acid and gas production in rabbits. In: 17. Internationale Tagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere, Celle, 11–12 May 2011.. Hoy S. (ed.). Gießen, VVB Laufersweiler Verlag: 138–149

Lavrenčič A., Stefanon B., Susmel P. 1997. An evaluation of the Gompertz model in degradability studies of forage chemical components. Animal Science, 64: 423–431

Makkar H.P.S., Singh B. 1987. Distribution of some hydrolitic and ammonia assimilation enzymes in gastrointestinal tract of rabbits. Nutrition Reports International, 36, 4:867–877

Menke K.H., Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development, 28: 375–386

Nicodemus N., Pérez-Alba L., Carabaño R., De Blas C., Badiola I., Pérez De Rozas A., García J. 2004. Effect of level of fibre and level of ground of fibre sources on digestion and ileal and caecal characterization of microbiota of early weaned rabbits. In: 8th World Rabbit Congress, Puebla, Mexico, 7–10 Sept. 2004:: 928–929. http://world-rabbit-science.com/WRSA-Proceedings/Congress-2004-Puebla/Papers/Feeding-&-Nutrition/N-Nicodemus.pdf

Nizza A., Moniello G. 2000. Meat quality and caecal content characteristics of rabbit according to dietary content and botanical origin of starch. World Rabbit Science, 8, 1: 3–9

Padilha M.T.S., Licois D., Gidenne T., Carre B. and Fonty G. 1995. Relationships between microflora and caecal fermentation in rabbits before and after weaning. Reprodution, Nutrition, Development, 35: 375–386

SAS Institute Inc. 2001. The SAS System for Windows, Release 8.02. Cary, NC, USA.

Trocino A., Fragkiadakis M., Majolini D., Carabano R., Xiccato G. 2011. Effect of the increase of dietary starch and soluble fibre on digestive efficiency and growth performance of meat rabbits. Animal Feed Science and Technology, 165: 265–277