SUPPLEMENTATION OF RABBIT DIET WITH CHESTNUT WOOD EXTRACT: EFFECT ON *IN VITRO* GAS PRODUCTION FROM TWO SOURCES OF PROTEIN

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ABSTRACT

In vitro gas production kinetics of soybean meal (SBM) and sunflower meal (SFM) were determined using three different inocula prepared from caecum contents of 78-day old rabbits of Slovenian meat line SIKA. The first inoculum was prepared from caecum contents of rabbits fed diet supplemented with 0.5% of chestnut wood extract (CWE) in the form of powder (POWD), the second one from caecum contents of rabbits fed diet supplemented with 0.6% of CWE coated with plant oil (COAT), while the third inoculum was prepared from caecum contents of rabbits not supplemented with CWE (CONT). Gas productions were measured over 60 h of incubation and obtained gas volumes were modelled with Gompertz model. The total potential gas productions (parameter "B" of the Gompertz model) were higher ($P<0.05$) when SBM and SFM were incubated in POWD (147 and 108 ml/g DM for SBM and SFM) than in CONT (137 and 97 ml/g DM for SBM and SFM). The highest parameter B was obtained for SBM when COAT was used (170 ml/g DM), while for SFM the highest parameter B was obtained when POWD (108 ml/g DM) was used. When both substrates were incubated in POWD they had shorter time of maximum fermentation rate (TMFR: 10.0 and 7.4 h for SBM and SFM) and higher maximum fermentation rate (MFR: 4.73 and 6.06 ml/h for SBM and SFM) than when incubated in CONT (TMFRs of 16.0 and 12.0 h for SBM and SFM, respectively, and MFRs of 3.58 and 4.07 ml/h for SBM and SFM, respectively) or COAT (TMFRs of 18.4 and 12.2 h and MFRs of 2.96 and 4.39 ml/h for SBM and SFM, respectively). In the first 8 h of fermentation, higher ($P<0.05$) amounts of gas were produced from substrates incubated in POWD (44 ml for both SBM and SFM) than in CONT (24 and 20 ml for SBM and SFM, respectively) and COAT (34 and 27 ml for SBM and SFM, respectively). These results suggest that CWE fed as the powder reaches the caecum increasing the caecal microbial fermentation, while CWE coated with plant oils (COAT) decreases the activity of CWE in the caecum, especially in the first 8 hours of fermentation. The caecal microorganisms are assumed to degrade the coating around CWE only when the incubation in the caecum is long enough, allowing to CWE to increase the fermentation. This effect was more evident in the fermentation of SBM, suggesting that the crude protein (CP) content of substrates (515 vs. 388 g/kg DM for SBM and SFM, respectively) could affect the kinetics of *in vitro* fermentation.

Key words: Rabbits, *In vitro* gas production, Caecal fermentation, Proteins.

INTRODUCTION

In intensive rabbit breeding the mortality is often very high and lead to high economic losses (Cheeke, 1987). The predominant reasons of mortality in growing rabbits are digestion disturbances, which are in majority of cases the consequence of inadequate nutrition. The influence of nutrients on microbial activity in rabbit caecum is very important, because it is directly linked to the rabbit health. To stabilise the caecal microbial fermentation various feed additives could be used. Among these (poly)phenolics, especially tannins, have great potential. Published results showed that tannins influences microbial activity in caecum (Štruklec *et al.*, 1993), improve production results (Štruklec and Kermauner, 1994) and reduce mortality in rabbits (Štruklec *et al.*, 1993; Atta and Mouneir, 2005; Maertens and Štruklec, 2006).

In Slovenia and some other European countries the sweet chestnuts (*Castanea sativa* Mill.) wood extract (CWE), containing mostly hydrolysable tannins, is frequently used in intensive rabbit breeding to control digestive disturbances. Tannins form complexes with proteins (Mangan, 1988; McLeod, 1974), thus forming thin layer of nonsolvent proteins on the surface of intestinal mucous membrane, which protects brush border from microbial colonisation, appeases peristaltics in the case of inflammation and prevents the dehydration (Farmatan, 1998). Complexes between tannins are more or less stable; however tannins form also complexes with amino acids, polysaccharides, metal ions, vitamins, bacterial cell membranes and enzymes involved in protein and carbohydrate digestion (Makkar, 2003; Grm, 2006). Formation of these complexes can provoke negative effects in upper part of GIT: reduces digestibility of nutrients and harms mucous membrane of small intestine, especially when higher concentrations of tannins are used. On the contrary, in lower part of GIT tannins can have favourable effects, because they could directly affect the activity of microbes by binding on their cell membranes (Butter *et al.*, 1999; McSweeney *et al.*, 2001). To take advantage of positive effects and to reduce or eliminate the negative effects, tannins should be protected against digestive processes in the upper GIT, allowing them to react specifically in the large intestine. To investigate this hypothesis we fed tannins coated with plant oils (Polaris, France) to growing rabbits and used their caecum contents as inoculum to determine the *in vitro* caecal microbial activity.

The caecal microbial activity could be evaluated using *in vitro* gas production technique. This technique is frequently used to examine the activity of the GIT microflora using various inocula prepared from rumen fluid (Menke and Steingass, 1988; Lavrenčič and Stefanon, 2001), gastrointestinal tract and faeces of pigs (Bauer *et al.*, 2004; Bindelle *et al.*, 2006) and caecum contents of rabbits (Marounek *et al*., 1997, 2000; Calabro *et al.*, 1999; Lavrenčič, 2007a, b; Kermauner, 2007).
The aim of the study was to compare the *in vitro* gas production kinetics of two protein sources, soybean and sunflower meal, incubated in inocula prepared from caecum contents obtained from rabbits fed with diets supplemented with CWE in the form of powder and coated with plant oils.

MATERIALS AND METHODS

Substrates

Two protein feeds, soybean meal (SBM, 515 g CP/kg DM) and sunflower meal (SFM, 388 g CP/kg DM) were chosen because they are most commonly used as protein sources in rabbit diets. Both substrates were milled to pass 1 mm screen before the *in vitro* trial.

In vitro fermentation

Manipulations and selection of animals and the preparation of inoculum were performed according to the methods described by Calabro *et al.* (1999) and Lavrenčič (2007). Two New Zealand White rabbits (Slovenian meat line SIKA) were fed the commercial compound feed (Krka, Novo mesto, Slovenia; CONT), two animals were fed the same compound feed supplemented with 0.5% of CWE powder (POWD; commercial product Farmatan®, 75% of tannins, the rest are natural sugars), Tanin (Sevnica, Slovenia), while two animals received the compound feed supplemented with 0.6% of CWE (Tanin, Sevnica, Slovenia) coated (COAT) with plant oils (16% of oil, Polaris, France). Diets were offered *ad libitum* from weaning at 35 days of age. The animals were sacrificed at 78 days of age and the caeca were isolated by tying off the two extremities with nylon string to prevent movement of the digesta. The inocula were prepared mixing the two caecum contents of animals fed CONT, POWD or COAT compound feed. Further manipulations were performed according to Lavrenčič (2007).

Calculations and statistical analysis

The gas produced at different times of incubation was corrected for the amount of gas produced from blank samples at the corresponding times within each repetition and type of inoculum. Obtained values were also corrected for the dry matter (DM) contents of 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, 1994). From the estimated parameters of Gompertz model other parameters were calculated: maximum fermentation rate (MFR; ml/h), time of maximum fermentation rate (TMFR; h), the delay in fermentation at the start of incubation (LAG; hours) and volume of gas produced until 8 hours of incubation (Gas8). Data concerning fermentation kinetic parameters (parameters B, C, A, LAG, MFR, TMFR and Gas8) were tested for significance by analysis of variance using the Scheffe test (SAS, 1994) with the model were substrate and type of inoculum were fixed effects.

RESULTS AND DISCUSSION

The estimated parameters of *in vitro* gas production are reported in Table 1 where the main effects of substrate and type of inoculum are also presented. There were significant differences in fermentation kinetic parameters ($P<0.001$) according to the type of inoculum and substrate. The interactions between substrate and type of inoculum were also significant (except for parameter C). The total potential gas production (parameter B of Gompertz model) was the lowest when both substrates were incubated in CONT. On the contrary, Roth (2003) and Sivka and Lavrenčič (2007) incubated different substrates in inoculum prepared from rumen fluid and found that gas production decreased with increasing concentrations of CWE. However, Grm (2007) determined that *in vitro* growth and proteolytic activity of two species of rumen bacteria increased in the presence of CWE. These results are in accordance with our results, where the highest parameters B were obtained when SBM was incubated in COAT and when SFM was incubated in POWD.

Table 1: Parameters of the Gompertz model of soybean meal and sunflower meal

Substrate	Inoculum	B† (ml/g DM)	C†	A†
Soybean meal (SBM)	CONT	137^{c}	3.11^{c}	0.071^{d}
	POWD	147^{b}	2.36^{d}	0.088^{c}
	COAT	170^{a}	2.41^{d}	0.047^{e}
Sunflower meal (SFM)	CONT	97^{e}	3.92^{a}	0.114^{b}
	POWD	108^{d}	3.08^{c}	0.153^{a}
	COAT	99^{de}	3.39^{b}	0.120^{b}
RMSE§		5.3	0.124	0.0127
Statistical significance				
Substrate		***	***	***
Inoculum		***	***	***
Substrate × inoculum		***		**

† B = asymptotic amount 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 the level $P < 0.05$

§ = root mean square error

However, the parameter B is normally obtained only after prolonged incubation of substrates in the inocula. During these prolonged incubations the caecal microorganisms are able to degrade the coating around CWE (COAT), releasing it into the medium and allowing it to react with (remaining) substrate, microbial enzymes and/or cell walls. Such effect was evident especially for SBM and could be related to higher content of crude protein (CP) in SBM than in SFM (515 vs. 388 g/kg DM, respectively). In addition to the estimated parameters of gas production (parameters B, C and A of Gompertz model) the calculated parameters such as the delay of fermentation at the start of incubation (LAG), maximum fermentation rate (MFR), time of maximum fermentation rate (TMFR) and gas produced until 8 h of incubation (Gas8) help to describe better the fermentation pattern of the substrates (Table 2). All these parameters were significantly ($P<0.05$) affected by the substrate, type of inoculum and interaction between the substrate and the type of inoculum. Generally, when both protein substrates were incubated in POWD, the fermentation started earlier (the shortest TMFR and LAG) and was more intensive (the highest MFR and Gas8) than in CONT or COAT (Table 2). The higher activity of anaerobes growing in the presence of CWE was also confirmed by Grm (2007) who found increased *in vitro* proteolytic activity of two species of rumen bacteria incubated in the medium containing CWE.

Table 2: Lag phase (LAG), maximum fermentation rate (MFR), time of maximum fermentation rate (TMFR) and gas produced till 8 hours of incubation (Gas8) from soybean meal and sunflower meal

Substrate	Inoculum	LAG (h)	MFR (ml/h)	TMFR (h)	Gas8 (ml/g DM)
Soybean meal (SBM)	CONT	1.9[b]	3.58[d]	16.0[b]	24[d]
	POWD	-1.4[d]	4.73[b]	10.0[d]	44[a]
	COAT	-3.0[e]	2.96[e]	18.4[a]	34[b]
Sunflower meal (SFM)	CONT	3.2[a]	4.07[c]	12.0[c]	20[e]
	POWD	0.8[c]	6.06[a]	7.4[e]	44[a]
	COAT	1.8[b]	4.39[bc]	10.2[d]	27[c]
RMSE[§]		0.44	0.23	0.95	1.5
Statistical significance					
Substrate		***	***	***	***
Inoculum		***	***	***	***
Substrate × inoculum		***	**	***	*

[a,b,c,d] = means in columns with different superscripts are significantly different at $P < 0.05$

[§] = root mean square error

We suppose that CWE added to the rabbit diets as powder was not digested before the caecum, thus influencing the microbial activity in the caecum. On the contrary, coating of CWE with plant oils (COAT) neutralized the function of CWE, especially in the first 8 hours of fermentation, which correspond to normal retention time in caecum (Gidenne, 1997). The oil coating was probably destroyed by ceacal microorganisms with prolonged incubation and then the released CWE could affect caecal fermentation (see parameter B in Table 1). The effect of coating was stronger with the incubation of SBM than with the incubation of SFM which had similar fermentation kinetics parameters when incubated in COAT and CONT.

Roth (2003) incubated different protein substrates (soybean meal, rapeseed meal and peas) *in vitro* in the culture prepared from rumen fluid containing increasing amounts of CWE powder and noted that the LAGs and TMFRs prolonged and MFRs decreased. Similar results were obtained also by Sivka and Lavrenčič (2007), who incubated pure cellulose in the inoculum prepared from rumen fluid containing increasing amounts of CWE powder. On the contrary, in the present study LAGs and TMFRs of substrates incubated in POWD were always shorter than those incubated in CONT. We suppose that differences between the present study and those where rumen fluid was used (Roth, 2003; Sivka and Lavrenčič, 2007) can be explained by the fact that in studies with rumen fluid higher concentrations of CWE were used, and because in the present study CWE was subjected to digestive processes in the rabbit stomach and small intestine, thus its chemical composition and activity was changed. Another reason is that the number, type and activity of microorganisms in caecal content differ from those in rumen fluid.

CONCLUSIONS

The main purpose of coating the chestnut wood extract (CWE) was to achieve the protection of tannins against the changes due to the action of hydrochloric acid in the stomach and digestive enzymes in the small intestine. Protected CWE thus will exhibit its beneficial effects, such as control of potentially harmful microorganisms in the large intestine and caecum. However, we observed only moderate differences in fermentation pattern in the first few hours between substrates incubated in inocula prepared from caecum contents of animals receiving diets without CWE and those receiving coated CWE. This suggests that the method used for coating CWE was not appropriate. On the contrary, the supplementation of rabbit diets with CWE in the form of powder positively affected the gas production until 8 h of fermentation and shortened the lag phase and time of maximum fermentation rate, suggesting that CWE supplemented to the diet as powder reached the caecum and increased the activity of caecal microbes. The effect was evident especially for SBM and could be related to higher content crude protein (CP) in SBM than in SFM (515 vs. 388 g/kg DM, respectively).

REFERENCES

Atta A.H., Mouneir S.M. 2005. Evaluation of some medicinal plant extracts for antidiarrhoeal activity. *Phytotherapy Research, 19, 6, 481-485.*

Bauer E., Williams B., Bosch M.W., Voight C., Mosentin R., Verstegen M.W.A. 2004. Differences in microbial activity of digesta from three sections of the porcine large intestine according to *in vitro* fermentation of carbohydrate-rich substrates. *J. Sci. Food Agric., 84, 2097-2104*.

Bindelle J., Buldgen A., Boudry C., Leterme P. 2007. Effect of inoculum and pepsin-pancreatin hydrolysis on fibre fermentation measured by gas production technique in pigs. *Anim. Feed Sci. Technol., 132, 212-226.*

Butter N.L., Dawson J.M., Buttery P.J. 1999. Effects of Dietary Tannins on Ruminants. Secondary Plant Products: Antinutritional and Beneficial Actions in Animal Feeding. *Nottingham, Nottingham University Press, 51-72.*

Calabro S., Nizza A., Pinna W., Cutrignelli M.I. Piccolo V. 1999. Estimation of digestibility of compound diets for rabbits using the *in vitro* gas production technique. *World Rabbit Sci., 7, 197-201*.

Cheeke P.R. 1987. Rabbit Feeding and Nutrition. *Academic Press, Orlando, USA, 372 pp.*

Farmatan. 1998. Dodatek h krmi za preprečevanje drisk pri živalih. (Feed additive for diarrhoea prevention.) *Informative material. Sevnica, Tanin, kem. ind., 4 pp.*

Gidenne T. 1997. Caeco-colic digestion in the growing rabbit: impact of nutritional factors and related disturbances. *Livestock Prod. Sci., 51, 73-88.*

Grm S. 2006. Vpliv kostanjevega tanina na rast in encimske aktivnosti vampnih bakterij *Streptococcus bovis* in *Selemononas ruminantium*. (Influence of chestnut tannins on growth and enzyme activities of rumen bacteria *Streptococcus bovis* and *Selemononas ruminantium*.) *Graduation thesis, Univ. Ljubljana, Dept. Anim. Sci., Domžale, 62 pp.*

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. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle, 2007-05-9/10. Giesen, DVG, 79-87.*

Lavrenčič A. 2007a. 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. 2007b. Comparison of *in vitro* fermentation of different pectins in inocula prepared from caecotrophs and from the caecum content of rabbits. *In: 15. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle, 2007-05-9/10, Giesen, DVG, 88-95.*

Lavrenčič A., Stefanon B. 2001. Prediction of *in situ* degradability of dry matter and neutral detergent fibre of temperate forages with in vitro gas production in fibre fractions. *Zootec. Nutr. Anim., 27, 179-192.*

Lavrenčič A., Stefanon B., Susmel P. 1997. An evaluation of the Gompertz model in degradability studies of forage chemical components. *Anim. Sci., 64, 423-431.*

Maertens L. Štruklec M. 2006. Technical note: preliminary results with a tannin extract on the performance and mortality of growing rabbits in an enteropathy infected environment. *World Rabbit Sci., 14, 189-192.*

Makkar H.P.S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Rumin. Res., 49, 241-256.*

Mangan J.L. 1988. Nutritional effects of tannins in animal feeds. *Nutr. Res. Rev., 1, 209-231.*

Marounek M., Vovk S.J., Benda V. 1997. Fermentation patterns in rabbit caecal cultures supplied with plant polysaccharides and lactate. *Acta Vet. Brno, 67, 9-13*.

Marounek M., Skrivanova V., Duškova D. 2000. *In vitro* caecal fermentation of nitrogenous substrates in rabbits. *J. Agric. Sci., 135, 437-442.*

McLeod M.N. 1974. Plant tannins – their role in forage quality. *Nutr. Abstr. Rev., 44, 11, 803-815.*

McSweeney C.S., Palmer B., McNeill D.M., Krause D.O. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. *Anim. Feed Sci. Technol., 91, 83-93.*

Menke K.H, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Develop., 35, 375-386.*

Roth S. 2003. Reducing methane emission and optimising N-supply in ruminants by treating feeds with tannins. *Doctoral Dissertation, Aachen, Schaker Verlag, 155*.

Sivka U., Lavrenčič A. 2007. The effect of sweet chestnut extract (Farmatan®) on kinetics of *in vitro* cellulose fermentation. *Krmiva, 49, 127-132.*

SAS 1994. SAS/STAT User's guide, Release 6.03 edition. *SAS Institute, Cary, NC, USA.*

Štruklec M., Kermauner A. 1994. Krmni dodatki v prehrani kuncev. (Feed additives in rabbit nutrition). *In: Posvetovanje o prehrani domačih živali "Zadravčevi-Erjavčevi dnevi", Radenci, 1994-10-27/28, p. 159-167.*

Štruklec M., Kermauner A., Kavar T. 1993. Einfluß der Kastanientannine auf pH-Wert, Bildung von Flüchtigen Fettsäueren, NH_3-Gehalt und auf die Gesamtacidität im Blinddarm der Kaninchen. *In: 8. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle, 1993-10-20/21. Giessen, DVG, 148-154.*