

EFFECT OF TANNIN TYPE ON *IN VITRO* GAS PRODUCTION

HANA PROCHÁZKOVÁ^{1,2}, DAVID ZAPLETAL¹, LUDMILA KŘÍŽOVÁ³

¹ Department of Animal Breeding, Animal Nutrition & Biochemistry,
University of Veterinary Sciences Brno, Palackého tř. 1946/1, 612 42
Brno, Czech Republic

² Agrovýzkum Rapotín s.r.o., Výzkumníků 863, 788 13 Rapotín, Czech
Republic

³ Department of Biochemistry, Faculty of Science, Masaryk University,
Kotlářská 267/2, 611 37 Brno, Czech Republic

Corresponding email address: h23441@vfu.cz

ABSTRACT

The aim of this study was to determine the effect of different types of tannins on total gas production using in vitro methods using ANKOM Gas Production System. For our study we used 6 products containing tannins commonly available on the market, representing both groups of tannins in the same concentration. Hydrolyzable tannins were presented by the products: FermiTan Harvest Hill, TOP House and Tan Clar. Condensed tannins were presented by the products: Tannin MOX, Tannin FC and VITANIL B. The study using the Ankom Gas Production System was carried out according to Van Soest (1970) with a 5% addition of individual tannins. Incubation was carried out for 48 hours at 39 °C. In our case, there was no evidence of a reduction in the

amount of gas produced. Both types of tannins increased the amount of gas produced compared to the control sample containing only the standard. Condensed tannins increased gas production more than hydrolyzed tannins.

Keywords: tannins; in vitro; gas production

INTRODUCTION

Tannins belong to the polymeric phenolic compounds present in a number of plant species which is use as feed across agriculture throughout the world. They occur in whole trees, shrubs, but also in by-products of agricultural processing, in leaves and stems of forage crops, cereals and grains, fruits, galls, pods and others (Addisu, 2016). They are also found in food and, as a natural component of food, they affect both desirable and undesirable taste properties of food. In the case of beverages such as fruit juices, beer or wine, tannins are removed by clarification, in order to prevent the formation of turbidity and sediments due to binding to proteins (Food safety, 2024).

Tannins are divided into two groups - group of hydrolyzable tannins and group of condensed tannins. Hydrolyzable tannins are esters of gallic acid or ellagic acid and their derivatives. (Multimediaexpo, 2024). Their occurrence is highest in the tissues of dicotyledonous trees and herbs, and they are stored mostly in leaves (Haslam, 2007). Condensed tannins are non-hydrolyzable oligo- and polymeric proanthocyanidins, which are made up of catechin units and are formed by the condensation of monomeric flavanols (Food safety, 2024). Condensed tannins are not broken down by hydrolysis (Reed, 1995) and may or may not be soluble in water and organic solvents,

depending on their chemical structure and degree of polymerization (Addisu, 2016). Condensed tannins are usually more widespread. Many plants contain both types of tannins. But there are some types of plants that contain only hydrolyzable tannins. Hydrolyzable tannins are thought to be more toxic because they are broken down by microorganisms in the rumen into pyrogallol, which is a hepatotoxin and a nephrotoxin. On the other hand, condensed tannins are not absorbed, but can be incorporated into the mucosa of the gastrointestinal tract and reduce the absorption of other substances there (Lotfi, 2020; Patra – Saxena, 2010; Reed, 1995). Condensed tannins in feed compounds can provide a means to protect feed proteins from degradation in the rumen, thereby increasing the uptake of plant proteins in the small intestine, and this in turn affects animal performance (Piluzza et al., 2013). Polyphenols are the main source of dietary antioxidants and are easily absorbed in the intestine (Kumar – Goel, 2019).

In general, the effect of tannins depends not only on the type of tannin contained, but also on its concentration. In lower concentrations, tannins can have rather beneficial effects, not only on digestion, but on the overall health of ruminants, faster growth of the animal's live mass, higher milk yield, good response of the immune system, reproductive performance, fertility, wool production, resistance to gastrointestinal parasites, prevention bloat and by reducing methane production also on the environment (Aerts et al., 1999; Fonseca et al., 2023; Kelln et al., 2021; Kumar et al., 2014; Mergeduš et al., 2018; Naumann et al., 2017; Tedeshi et al., 2014; Yanza et al., 2024). In higher concentrations, tannins have rather harmful effects on feed intake, rumen microflora, use of nutrients received and overall their production.

MATERIAL AND METHODS

For our study we used 6 products containing tannins, representing both groups of tannins in the same concentration. Hydrolyzable tannins presented by the products: FermiTan Harvest Hill (ellagic tannin from selected wood species), TOP House (fine tannin from ground opal wood from French oak) and Tan Clar (pure hydrolyzed ellagic tannin) (Picture 1).



Picture 1. Selected products containing hydrolyzabled tannins

Condensed tannins presented by: Tannin MOX (condensed tannin of the Quebraco tree), Tannin FC (tannin containing catechin, used to support anthocyanin and polymerize into stable polymers) and VITANIL B (tannin from gall alcohol) (Picture 2).



Picture 2. Selected products containing condensed tannins

The study of apparent digestibility using the ANKOM Gas Production System was carried out according to Van Soest (1970). As inoculum we used a mixture of rumen fluids from 3 cannulated cows. Ruminal fluid was collected 2 hours after morning feeding into pre-tempered thermoses. To each 250 ml glass bottle with septum, was prepared 20 ml of inoculum, 80 ml of final buffer and a weighed 1 g sample of the standard together with a 5% tannin sample. After removing oxygen, the glass bottles were incubated in a thermostat for 48 hours at 39 °C. The temperature and pressure of the gas in the bottles were monitored.

Data has been processed by Microsoft Excel.

RESULTS AND DISCUSSION

From the Table 1 is evident that both types of tannins increased the amount of gas produced compared to the control sample containing only the standard. Condensed tannins increased gas production more than hydrolyzed tannins.

Table 1. Amount of *in vitro* gas production in individual products

		Amount of gas (ml)	Percentage differences
Group 1	Control	1319.42	
	Fermitan	1350.21	increase of 2.33 %
	TOP House	1420.58	increase of 7.67 %
	Tan Clare	1345.81	increase of 2 %
Group 2	Control	1402.99	
	MOX	1464.56	increase of 4.39 %
	FC	1499.75	increase of 6.9 %
	VITANIL B	1627.29	increase of 15.99 %

The results of our study do not agree with the results of studies conducted by other authors. A moderate concentration of tannins in animal food destabilizes protein foams, which makes them safe against flatulence (Kumar et al. 2014, Mergeduš et al. 2019). According to Aerts et al. (1999) it is well documented that bloat occurs when grazing ruminants consume large amounts of legumes (alfalfa or clover for example). Gases produced in the rumen during fermentation cannot be released in the normal way because they are trapped in the persistent foam caused by the rapid release of soluble proteins during chewing and ruminal degradation. However, when these animals graze on legumes containing condensed tannins (such as *Onobrychis viciifolia*), entrapment does not occur and gases can escape from the digestive tract.

In contrast, Getachew et al. (2008) point out that the addition of tannin had no effect on the amount and rate of gas production but significantly reduced the concentration of ammonia nitrogen. Fagundes et al. (2020)

points to digestibility when, in their study, with the addition of condensed tannins, there was no reduction in nutrient digestibility, nor a deterioration in fermentation parameters (certain types of archaea were suppressed, but overall the number of bacteria in the rumen increased).

For our study, the rumen fluid of dry-resistant crossbred meat breed cows commonly raised in the Czech Republic was used as inoculum. Bueno et al. (2020) in their study showed on the different results of gas production according to the use of different types of inoculum (Holstein dairy cows, Nelore beef cattle, Mediterranean water buffalo, Santa Inês sheep and Saanen goats). Therefore, the results of our study may not be applicable to all ruminants.

CONCLUSION

The study carried out by us opens another field for the study of suitable preparations but also individual concentrations, or combinations of preparations not only to reduce the total volume of gas produced, but also to distinguish individual gases produced and possible reduction of both total and ammonia emissions.

However, for a global solution, it will be necessary to conduct a study not only on cattle as representatives of ruminants, but to conduct a study on several species of ruminants.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO1224.

REFERENCES

- Addisu S. (2016): Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; a review. *Online Journal of Animal and Feed Research*, vol. 6, issue 2, p. 45-56.
- Aerts R. J., Barry T. N., McNabb W. C. (1999): Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agriculture, Ecosystems and Environment*, vol 75, p. 1-12.
- Bueno I.C.S., Brandi R. A., Fagundes G. M., Benetel G., Muir J. P. (2020): The Role of Condensed Tannins in the In Vitro Rumen Fermentation Kinetics in Ruminant Species: Feeding Type Involved? *Animals*, vol. 10, 635.
- Fagundes G. M., Benetel G., Welter K. C., Melo F. A., Muir J. P., Carriero M. M., Souza R. L. M., Meo-Filho P., Frighetto R. T. S., Berndt A., Bueno I. C. S. (2020): Tannin as a natural rumen modifier to control methanogenesis in beef cattle in tropical systems: friend or foe to biogas energy production? *Research in Veterinary Science*, vol. 132, p. 88-96.
- Food safety (2024): Trisloviny. [Online] [Cited: 20. 5. 2024]. Available from: <https://bezpecnostpotravin.cz/termin/trisloviny/>
- Fonseca N. V. B., Da Silva Cardoso A., De Souza Bahia A. S. R., Messana J. D., Vicente E. F., Reis R. A. (2023): Additive Tannins in Ruminant Nutrition: An Alternative to Achieve Sustainability in Animal Production. *Sustainability*, vol. 15, 4162.
- Getachew G., Pittroff W., DePeters E. J., Putnam D. H., Dandekar A., Goyal S. (2008): Influence of tannic acid application on alfalfa hay: in vitro rumen fermentation, serum metabolites and nitrogen balance in sheep. *Animal*, vol 2:3, p. 381-390.
- Goering H. K. and Van Soest P. J. (1970): Forage fiber analysis (apparatus, reagents, procedures and some applications), *Agricultural Handbook No. 379 ARS-USDA*, Washington, DC.
- Haslam E. (2007): Vegetable tannins – Lessons of a phytochemical lifetime. *Phytochemistry*, vol. 68, 2713-2721.
- Kelln B. M., Penner G. B., Acharya S. N., McAllister T. A., Lardner H. A. (2021): Impact of condensed tannin-containing legumes on ruminal fermentation, nutrition, and performance in ruminants: a review. *Canadian Journal of Animal Science*, vol. 101, p. 210-223.
- Kumar K., CHaudhary L. C., Kumar S. (2014): Exploitation of tannins to modulate rumen ecosystem and ruminants performance: A review. *Indian Journal of Animal Sciences*, vol. 84 (6), p. 609-618.

- Kumar N., Goel N. (2019): Phenolic acid: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, vol. 24, e00370.
- Lotfi R. (2020): A commentary on methodological aspects of hydrolysable tannins metabolism in ruminant: a perspective view. *Letters in Applied Microbiology*, vol. 71, p. 466-478.
- Mergeduš A., Pšenková M., Brus M., Janžekovič M. (2018): Tannins and their Effect on Production Efficiency of Ruminants. *Agricultura*, vol. 15, no. 1-2, p. 1-11.
- Multimediaexpo (2024): Třísloviny. [Online] [Cited: 20. 5. 2024]. Available from: <http://www.multimediaexpo.cz/mmecz/index.php/Třísloviny>
- Naumann H. D., Tedeschi L. O., Zeller W. E., Huntley N. F. (2017): The role of condensed tannins in ruminant animal production: advances, limitations and future directions. *Revista Brasileira de Zootecnia*, vol. 46 (12), p. 929-949.
- Patra A. K., Saxena J. (2010): Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.*, vol. 91, p. 24-37.
- Pilluzza G., Sulasand L., Bullitta S. (2013): Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. John Wiley & Sons Ltd. *Grass and Forage Science*, vol. 69, p. 32-48.
- Reed J. D. (1995): Nutritional Toxicology of Tannins and Related Polyphenols in Forage Legumes. *Journal of Animal Science*, vol. 73, p. 1516-1528.
- Tedeschi L. O., Ramírez-Restrepo C. A., Muir J. P. (2014): Developing a conceptual model of possible benefits of condensed tannins for ruminant production. *Animal*, vol. 8:7, p. 1095-1105.
- Yanza Y. R., Irawan A., Jayanegara A., Ramadhani F., Respati A. N., Fitri A., Hidayat C., Niderkorn V., Cieslak A., Szumacher-Strabel M., Hidayat R., Tanuwiria U. H. (2024): Saponin Extracts Utilization as Dietary Additive in Ruminant Nutrition: A Meta-Analysis of In Vivo Studies. *Animals*, vol. 14, 1231.