Effect of Tannin Content in Horse Bean on Rumen Fermentation *in vitro*

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Abstract

The objective of the experiments was to demonstrate the influence of low- and high-tannin horse bean on the selected fermentation indicators. The sheep rumen content with the addition of 1, 2 and 5 g of high- and low-tannin horse bean was incubated in vessels of 250 ml volume. A significant increase of ammonia, lactic acid, CO_2 and methane as well as pH decrease in samples with addition of horse bean seed was demonstrated. While comparing high- and low-tannin horse bean varieties, it was observed that in the case of small doses of 1 and 2 g a higher intensity of fermentation takes place in the samples with the addition of high-tannin horse bean. In the case of larger doses of 5 g per sample, a higher intensity of fermentation takes place in the samples with the addition of low-tannin horse-bean. A probable reason for the decrease in the fermentation process intensity in the samples with the addition of 5 g of high-tannin horse bean is a high content of tannins, which reduce fermentation processes.

Sheep, fermentation, rumen, horse bean, in vitro

A ban on the inclusion of meat-bone meal in the ruminant feeding has resulted in a greater interest of breeders in high-protein plant fodders. These fodders are considerably cheaper than the meat-bone meal and do not involve a risk of infectious diseases. One disadvantage of the fodders is worse protein balance in the amino-acid composition. Horse bean belongs to this type of fodders. Its high nutritive value is associated with a high protein content of high biological value. The horse bean seeds contain approximately 26-30% of the total protein. Globulins are the main protein, which constitutes 55% to 70% of the total protein (Jasińska and Kotecki 1998a; 2003). A disadvantage of the protein contained in the horse bean is the low content of some amino acids, i.e. methionine, cysteine, tryptophan and threonine, whereas its content of lysine is high (Jasińska and Kotecki 1993). The fat content in the horse bean seeds is very low. The horse bean also contains a substantial amount of sugar, including a lot of starch and hemicellulose amounting to approximately 58%. It is also rich in minerals and vitamins (Jasińska and Kotecki 2003; Pavlata et al. 2002). There are certain compounds in the horse bean that limit the bioavailability of nutritive components and disturb the functions of glands and internal organs through which they worsen health and reduce animal productivity. The following compounds belong to the above-mentioned ones: tannins, protease inhibitors, lectins, glycosides, phytates, alpha-amylase and oligosaccharide inhibitors (Jasińska and Kotecki 1998b).

Tannins are intermediate metabolites of the plant synthesis. They are structurally divided into two groups: proanthocyanidins (the condensed tannins) and polyesters of the gallic acid and the hexahydroxydiphenic acid (Hasam 1981). Tannins have the task of protecting the plants against herbivorous animals and those microorganisms that are pathogenic to plants (fungi, bacteria) (Provenza 1995; Scalbert 1991; Hart and Hillis 1972). Tannins have an impact on decreasing the animal's appetite, which already appears 20-60 min after administration of the horse bean fodder (a short-term effect). The addition of the horse

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bean in the fodder has also a long-term effect on a decrease in the feed consumption (a long-term effect). The short-term effect is associated with changes within the oral cavity, whereas the long-lasting feeding results in a decrease in the concentration of ammonia and volatile fatty acids in the rumen contents. In the long-term effect, the fermentation process in the rumen is limited and a decrease in energy is provided for the animal (Silanikove et al. 2001). On account of the tannin content in the horse bean seeds, different horse bean varieties have been divided into low- and high-tannin ones. In the low-tannin varieties, the content of condensed tannins in the coated seeds does not exceed 0.04 mg/g dry mass of the seeds. The low-tannin varieties studied in this work include the following varieties (contents of tannin are given in parentheses in milligrams per gram of dry mass): Allbus (0.034 mg/g d. m.), Leo (0.026 mg/g d. m.), Olga (0.038 mg/g d. m.), Kasztelan (0.027 mg/g d. m.), Merlin (0.031 mg/g d. m.), and Mistral (0.021 mg/g d. m.). The high-tannin varieties were as follows: Akord (0.594 mg/g d. m.), Martin (0.725 mg/g d. m.), Neptun (0.750 mg/g d. m.), Optimal (0.755 mg/g d. m.), Redos (0.689 mg/g d. m.), and Sonet (0.716 mg/g d. m.).

The current knowledge of feeding of horse bean in ruminants is unsatisfactory and more data are needed.

Materials and Methods

The study was carried out on 15 sheep, (Polish Merino × Polish Heather Sheep hybrid at the age from 2 to 4 years and of the body mass of 30-45 kg) from which samples of the rumen fluid and contents were taken 2.5 h after the morning feeding. The sheep were fed hay and concentrate mixture J-161 DB411X manufactured by Provimi-Rolimpex S.A.

The material for analysis was collected through the cannule of the dorsal rumen sack, made in accordance with Dejneka and Zięba's method (1965). Incubation of the collected contents was conducted in the vessels in accordance with Barnett and Reid's method (1961) with Zawadzki's modification (1993). The incubation environment in the vessels included 50 ml of the collected rumen fluid contents, 50 ml of distilled water and 50 ml of McDougall's artificial saliva of pH = 6.9 with the following composition (in 1 dm^3 of distilled water): $NaHCO_3$ – 9.60 g, KCl – 0.60 g, $CaCl_2$ – 0.04 g, $Na_2HPO_4 \cdot 12H_2O$ – 9.15 g, $NaCl$ – 0.45 g, $MgSO_4 \cdot 7H_2O$ – 0.11 g, $ZnSO_4 \cdot H_2O$ – 0.06 g, $CaCl_2 \cdot 6H_2O$ – 0.01 g, NH_4HCO_3 – 0.5 g. A control sample consisted of vessels including 50 ml of the collected contents, 50 ml of distilled water and 50 ml of artificial saliva. A control sample was prepared individually for each experiment in order to eliminate differences in the fermentation activity of the rumen contents collected. The next vessels included 50 ml of the filtered rumen contents, 50 ml of distilled water and 50 ml of artificial saliva as well as ground seeds of the low- and high-tannin horse bean at 3 different doses of 1, 2 and 5 g. Twenty fermentations were performed for each variant. The doses of 1, 2 and 5 g were adopted from previous research. The total incubation volume of the samples in the vessels amounted to 150 ml (with the total vessel volume of 250 ml). The vessel was made leak-proof by polished plugs and valves, covered with sealing silicon lubricant. Nitrogen was added to vessels under pressure to obtain anaerobic conditions. The gas was passed through the vessel contents for 10-15 min. Then, all the valves were closed. The maintaining of anaerobic conditions was checked with a DO-5508 device (manufactured by Lutron). The vessels prepared in this way were then placed in the seats of a shaker with a water bath of 39-42 °C and amplitude of 4 cycles per min. During incubation, samples of the contents were collected at the 4th and 6th hour of incubation in order to determine the content of ammonia, lactic acid, methane, CO_2 as well as the pH of the fermented rumen contents. The ammonia level was determined using the Conway method, and the lactic acid and CO_2 concentration was determined using the titration method. Methane concentration was measured using a methanometer of Barbara-3 type. The pH of the collected samples of the incubated rumen contents was measured with a pH-meter (pX-processor PM-600). The research results were elaborated statistically by Student's t-test and the ANOVA variation analysis.

The experiment was conducted in accordance with protocol approved by the II Local Ethics Commission located at the Wroclaw Uviwersity of Enviromental and Life Sciences Poland.

Results

Changes of ammonia concentration

The addition of the low-tannin horse bean to the rumen contents fermented in *in vitro* conditions resulted in significant intensification of the fermentation processes in the rumen contents, especially the protein decomposition processes. A significant increase in ammonia concentration was noticed compared to the values obtained in the control sample. Bacterial fermentation of the horse bean seeds occurs very quickly, and ammonia concentration in

Table 1. Values of the selected fermentation indicators of the sheep rumen contents with the addition of the low-tanin horse bean

Addition weight	Time of sample collection	Ammonia (mmol/l)	Lactic acid (mmol/l)	pH	CO_2 (mol/l)	Methane (% vol.)
1 g	4 h	4.49 ± 0.61**	2.33 ± 0.46*	7.82 ± 0.05	0.00018 ± 7.07•10⁶*	1.975 ± 0.25
	6 h	5.59 ± 0.45**	3.33 ± 0.18	8 ± 0.081		2.025 ± 0.28
2 g	4 h	5.98 ± 1.45**	3.66 ± 0.38**	7.87 ± 0.095	0.000217 ± 2.21•10⁵***	2.2 ± 0.01
	6 h	7.32 ± 1.22***	4.21 ± 0.24**	7.87 ± 0.095		2.05 ± 0.17
5 g	4 h	8.99 ± 0.45***	3.99 ± 0.24**	7.77 ± 0.17	0.00035 ± 8.16•10⁶***	2.075 ± 0.05
	6 h	15.71 ± 0.86***	8.21 ± 0.53***	7.72 ± 0.15		2.1 ± .015
Control sample	4 h	2.26 ± 0.21	1.77 ± 0.09	8.47 ± 0.05	0.00013 ± 2.88•10⁵	1.85 ± 0.5
	6 h	2.4 ± 0.43	2.99 ± 0.27	8.42 ± 0.095		1.7 ± .01

  * significant increase in the value compared to the control sample $p < 0.05$
 ** significant increase in the value compared to the control sample $p < 0.01$
*** significant increase in the value compared to the control sample $p < 0.005$

the rumen contents fermented with the addition of 1 g of the low-tannin horse bean was higher by 98.7% ($p < 0.01$) on average at the 4th hour; with the addition of 2 g it was higher by 164.93% ($p < 0.01$); and with the addition of 5 g it was higher by 595.32% ($p < 0.005$) compared to the values obtained in the control sample (Table 1). At the 6th hour of the fermentation, ammonia concentration increased by 132.19% ($p < 0.01$) in samples with the addition of 1 g of the low-tannin horse bean; with the addition of 2 g it was higher by 204.14% ($p < 0.005$); and with the addition of 5 g it was higher by 552.92% ($p < 0.005$) compared to ammonia concentration determined for the control sample at the 6th hour of fermentation.

The addition of the high-tannin horse bean results in an increase of ammonia concentration by 255.07% ($p < 0.01$) with the addition of 1 g of the horse bean at the 4th hour of incubation; by 392.75% ($p < 0.01$) with the addition of 2 g; and by 386.08% ($p < 0.005$) with the addition of 5 g of the horse bean. At the 6th hour of fermentation, ammonia concentration increased by 131.35% ($p < 0.05$) with the addition of 1 g of the horse bean; by 278.3 ($p < 0.005$) with the addition of 2 g; and by 307.96% ($p < 0.005$) with the addition of 5 g (Table 2).

Table 2. Values of the selected fermentation indicators of the sheep rumen contents with the addition of the high-tanin horse bean

Addition weight	Time of sample collection	Ammonia (mmol/l)	Lactic acid (mmol/l)	pH	CO_2 (mol/l)	Methane (% vol.)
1 g	4 h	7.19 ± 0.94**	0.033 ± 0.0023**	8 ± 0.05	0.00024 ± 4.54•10⁵*	2.17 ± 0.05
	6 h	8.01 ± 1.08*	0.038 ± 0.0030*	8 ± 0.05		2 ± 0.23
2 g	4 h	9.98 ± 0.63*	0.039 ± 0.053**	7.97 ± 0.05	0.00028 ± 2.98•10⁵***	1.85 ± 0.1
	6 h	13.10 ± 1.41***	0.063 ± 0.015**	7.87 ± 0.15		1.9 ± 0.2
5 g	4 h	9.84 ± 0.47***	0.05 ± 0.0067**	7.8 ± 0.14	0.00037 ± 3.20•10⁵***	2.1 ± 0.081
	6 h	24.07 ± 1.76***	0.068 ± 0.010***	7.7 ± 0.01		2.025 ± 0.15
Control sample	4 h	3.45 ± 0.17	0.018 ± 0.00061	8.52 ± 0.05	0.00014 ± 0.5•10⁵	1.9 ± 0.05
	6 h	5.9 ± 0.18	0.027 ± 0.0025	8.5 ± 0.01		1.25 ± 0.63

  * significant increase in the value compared to the control sample $p < 0.05$
 ** significant increase in the value compared to the control sample $p < 0.01$
*** significant increase in the value compared to the control sample $p < 0.005$

Comparing ammonia concentration in the samples that included the low-tannin horse bean and the samples with the addition of the high-tannin horse bean, a certain relation can be observed. The addition of the high-tannin horse bean caused a higher increase of ammonia concentration in the fermented contents when added at low doses of 1 and 2 g. At the 4th hour of fermentation, ammonia concentration in samples with ground high-tannin horse bean seeds was higher by 58.6% ($p < 0.01$) when 1 g was added and by 66.66% ($p < 0.01$) when 2 g of seeds were added compared to the values obtained in samples with the low-tannin horse bean. At the 6th hour of fermentation, ammonia concentration was higher by 43.38% ($p < 0.05$) in samples with the addition of 1 g of the high-tannin horse bean and by 78.98% ($p < 0.01$) with the addition of 2 g of seeds compared to the values obtained in the samples with the low-tannin horse bean. When a higher dose of 5 g of the high-tannin horse bean was added, higher concentration of ammonia in samples with the high-tannin horse bean was no longer observed.

At the 4th hour of fermentation, the ammonia concentration in the samples with the high-tannin horse bean was higher by 9.46% (not a significant change) compared to the values obtained in the samples with the low-tannin horse bean. However, at the 6th hour of fermentation, the ammonia concentration in the case of the high-tannin horse bean was lower by 10.8% ($p < 0.05$) than in the case of the low-tannin horse bean.

Changes of the lactic acid concentration

The addition of the low-tannin horse bean resulted in an increase of lactic acid concentration in the determined sample by o 31.25% ($p < 0.05$) with the addition of 1 g of the horse bean at the 4th hour of fermentation; by 106.25% ($p < 0.01$) with the addition of 2 g of the horse bean; and by 125% ($p < 0.01$) with the addition of 5 g of the seeds compared to the lactic acid concentration in the control sample (Table 1). At the 6th hour of fermentation, lactic acid concentration increased by 11.11% with the addition of 1 g (not a significant increase), by 40.74% ($p < 0.01$) with the addition of 2 g, and by 174.07% ($p < 0.005$) with the addition of 5 g of the horse bean seeds compared to the lactic acid concentration in the control sample.

The addition of high-tannin horse bean seeds caused a much higher increase in lactic acid concentration than low-tannin horse bean seeds. At the 4th hour of fermentation, the increase of lactic acid concentration after the addition of 1 g of high-tannin horse bean seeds to the examined sample amounted to 83.33% ($p < 0.01$), whereas the addition of 2 g and 5 g resulted in an increase in concentration by 116.66% ($p < 0.01$) and 177.77% ($p < 0.01$), respectively. At the 6th hour of fermentation, increases of lactic acid concentration amounted to: 40.74% ($p < 0.05$) with the addition of 1 g, 133.33% ($p < 0.01$) with the addition of 2 g, and 151.85% ($p < 0.005$) with the addition of 5 g of the horse bean seeds (Table 2).

Comparison of lactic acid concentrations obtained in samples with the low- and high-tannin horse bean, the addition of the high-tannin horse bean results in noticeably higher increases of lactic acid concentration when small doses of 1 and 2 g are added than the same addition of the low-tannin horse bean. The addition of the high-tannin horse bean at higher doses results in lower increases of lactic acid concentration compared to the low-tannin horse bean.

Changes of CO_2 concentration

The addition of the low-tannin horse bean at the 4th hour of fermentation causes an increase in CO_2 concentration by 38.46% ($p < 0.05$) when 1 g of the horse bean seeds is added; by 66.92% (p < 0.01) with the addition of 2 g; and by 169% ($p < 0.01$) with the addition of 5 g compared to the values obtained in the control samples.

The addition of the high-tannin horse bean at the 4th hour of fermentation causes an increase in CO_2 concentration by 98.26% ($p < 0.05$) when 1 g of the horse bean seeds is

added; by 100% ($p < 0.01$) with the addition of 2 g; and by 164.28% ($p < 0.01$) with the addition of 5 g compared to the values obtained in control samples.

Comparing CO_2 concentration obtained in samples with the low- and high-tannin horse bean, it can be observed that the addition of the high-tannin horse bean at small doses of 1 and 2 g caused a higher increase of CO_2 concentration by 122.22% and 29.03%, respectively, than the addition of the same amount of the low-tannin horse bean. The addition of the high-tannin horse bean at higher doses results in a lower increase of CO_2 concentration (only by 5.71%) compared to the low-tannin horse bean.

Changes of the methane concentration

The addition of the low-tannin horse bean at the 4th hour of fermentation causes an increase in methane concentration by 6.75% when 1 g of the horse bean seeds is added; by 18.91% with the addition of 2 g; and by 11.89% with the addition of 5 g compared to the values obtained in the control samples.

At the 6th hour of fermentation, increases of methane concentration amounted to: 19.11% with the addition of 1 g; 20.58% with the addition of 2 g; and 23.52% with the addition of 5 g of the horse bean seeds compared to methane concentration in the control sample.

The addition of the high-tannin horse bean at the 4th hour of fermentation caused an increase in methane concentration by 14.21% when 1 g of the horse bean seeds is added; a decrease by 2.63 % with the addition of 2 g; and an increase by 10.62% with the addition of 5 g compared to the values obtained in control samples. At the 6th hour of fermentation, the increases of methane concentration amounted to: 60% with the addition of 1 g; 52% with the addition of 2 g; and 61.6% with the addition of 5 g of the horse bean seeds.

Comparing methane concentration obtained in samples with the low- and high-tannin horse bean, it can be observed that the addition of the high-tannin horse bean generally caused a decrease in methane concentration. Comparing methane concentration obtained in samples with the addition of the low- and high-tannin horse bean at a dose of only 1 g per sample at the 4th hour of fermentation, an increase in methane concentration by 10.15% was obtained when the high-tannin horse bean was added. However, when the doses of 2 and 5 g were added, methane concentration decreased by 15.9% and 2.38%, respectively. At the 6th hour of fermentation, methane concentrations lower by 2.43% (at the dose of 1 g), 7.31% (at the dose of 2 g) and by 3.8 (at the dose of 5 g) were observed in samples with the high-tannin horse bean than with the same addition of the low-tannin horse bean.

Changes of pH

The addition of the low-tannin horse bean to the fermented contents caused a decrease of the pH of the fermented sample by 7.67% when 1 g of the horse bean seeds was added at the 4th hour of the fermentation; by 7.08% with the addition of 2 g; and by 8.26% with the addition of 5 g compared to the values obtained in control samples. At the 6th hour of fermentation, a comparable decrease in the pH of the fermented contents was noted, by: 5.25% with the addition of 1 g; 6.53% with the addition of 2 g; and 8.31% with the addition of 5 g of the horse bean seeds.

The addition of the high-tannin horse bean at the 4th hour of fermentation causes a decrease of the pH of the fermented contents by 6.1% with the addition of 1 g of the horse bean seeds; by 6.45% with the addition of 2 g, and by 8.45% with the addition of 5 g compared to the values obtained in control samples. At the 6th hour of fermentation, the registered decrease in the pH of the fermented contents was comparable with the decrease noted at the 4th hour and amounted to: 5.88% with the addition of 1 g; 7.41% with the addition of 2 g; and 9.41% with the addition of 5 g of the horse bean seeds.

Comparing the changes of pH values of the fermented contents obtained in samples with the addition of the low- and high-tannin horse bean, it can be observed that the decrease of the pH of the fermented contents was comparable for both cases.

Discussion

Horse bean seeds are a valuable fodder supplement in ruminant feeding for their high protein content. Protein contained in the horse bean seeds is very valuable and is subject to quick decomposition in the rumen contents, intensifying the rumen fermentation processes as confirmed by this work. The addition of only 1 g of the horse bean results in a significant increase in the concentration of ammonia, lactic acid, CO_2, methane, and a decrease in the pH of the fermented contents compared to control samples. The ammonia concentration in the samples with the addition of the low-tannin horse bean grows by nearly 100% already at the dose of 1 g. Higher doses cause proportionally higher increases in ammonia concentration. Changes of lactic acid concentration in samples with the addition of horse bean are equally high. Intensification of the fermentation process observed in samples with the addition of horse bean seeds also manifests itself as an increase in the fermentation gases, CO_2 and methane. The increase in CO_2 concentration is significant and is as high as the increase in the concentration of ammonia and lactic acid, reaching 100% with the addition of 1 g of the high-tannin lupin seeds. However, increases of methane concentration observed for the addition of the low-tannin horse bean are considerably lower, reaching a maximum of 23.52%, but only at the 6th hour of fermentation. At the same hour of fermentation, analogous changes of ammonia and lactic acid concentrations considerably exceeded 100%. Only in the case of the high-tannin horse bean the increase of methane was slightly higher and amounted to approximately 60% at the 6th hour of fermentation. The observed decrease in the pH of the fermented contents is caused by the increase of the concentration of volatile fatty acids, which is due to the process of rumen contents. Intensification of fermentation is caused by the addition of the horse bean seeds to the fermented samples, which gives the animal valuable extra energy and amino acids necessary for the correct functioning of the organism.

Considering the differences between the low-tannin and high-tannin horse bean, it can be observed that at low doses of 1 and 2 g, the addition of the high-tannin horse bean causes greater intensification of the fermentation process in relation to the analogous addition of the low-tannin horse bean. This is manifested by higher increases of the concentration of ammonia, lactic acid, CO_2 and methane. However, when a higher dose of 5 g per sample is added, an opposite tendency can be observed. At higher doses, the addition of the high-tannin horse bean causes lower increases of the concentration of selected indicators than analogous addition of low-tannin horse bean seeds. The high-tannin horse bean in high doses limited the fermentation process resulting from the presence of the additional amount of protein, fat, and mineral compounds contained in the horse bean. Limitation of the fermentation process in the case of addition of the high-tannin horse bean at high doses is likely to result from a high content of tannins, which is proved by the results obtained by Silanikove et al. (1994; 1996; 1997). The authors pointed out that the anti-nutritional impact of tannins consisted of a reduction of bioavailability of the protein contained in the fodder as well as lowering of the activity of the enzymes secreted in the digestive tract; the pancreatic enzymes are particularly sensitive and their enzymatic activity is subject to considerable lowering in sheep and goats (Silanikove et al. 1994; Silanikove et al. 1996). Tannins considerably reduce digestibility of the plant cell walls through bonding with bacterial enzymes or forming indigestible complex compounds with carbohydrates of the cell walls (Barry et al. 1986; Reed 1986).

High tannin concentration significantly worsens protein digestibility as proved by numerous studies. In the *in vitro* research consisting of the incubation of bean (Barroga et. al. 1995) and horse bean (Carrido et al. 1991) seeds in a solution containing numerous enzymes, it was found that the presence of tannins decreased the decomposition of protein

performed by enzymes. The research carried out by McNabb et al. (1998) examining the influence of tannins on solubility and degradation of the protein from *Lotus pedunculatus* by the carboxylaze enzymes from the rumen fluid, confirms the negative influence of tannins on protein decomposition. Research using leguminous plant seed fermentation with or without the addition of the substances that bind tannins also presented an important proof of the negative impact of tannins on the use of protein (Makkar et al. 1995; McSweeney et al. 1999; Palmer and Jones 2000; Jones et al. 2000). The addition of substances that bind tannins or proper heat treatment result in an increase of fermentation, which is manifested by a greater volume of gases created during rumen fermentation. The *in vivo* experiments carried out on ruminants also prove the influence of tannins on the extent of fodder digestibility. Putting a nylon or polyester sack filled with ground leguminous plant seeds in the rumen through the cannule of the dorsal rumen sack, which was made earlier, allowed to propose a thesis that tannins limit the use of nutrients in the fodder through limiting the decomposition processes. However, the addition of binding substances (PEG-water-soluble polyethylene glycol) prevents this process (Aharoni et al. 1998). This enables the breeder to obtain better economic results in breeding through a better use of fodder by the animals (Silanikove et al. 1997; Ben Salem et al. 2000). Research on goats fed oak leaves (that are rich in tannins) with the addition of soya seeds and PEG, the tannin-binding substances PEG, pointed out that the addition of PEG caused an increase in raw protein digestibility from 92 to 122 g per day. Consumption of the fodder also increased from 844 to 1,023 g per day (Silanikove et al. 1997). Tannins also have a direct impact on the fermentation activity of rumen microorganisms by lowering this activity (Silanikove et al. 1997).

This experimental model is widely used in the research into digestibility, providing much valuable information as the fodder decomposition processes occur in the natural environment of the rumen (Orskov and McDonald 1979; Khazaal 1993).

The addition of both the high-tannin and low-tannin horse bean intensifies rumen fermentation processes. The addition of the high-tannin horse bean at lower doses of 1 and 2 g causes higher increases of the rumen fermentation indicators under study. When higher doses of the high-tannin horse bean (5 g) are added, no differences in the examined fermentation indicators can be observed or, alternatively, a decrease in the indicators is noticed.

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