Journal of Elementology ISSN 1644-2296

Coşkuntuna, L., Erten, K. and Şamlı, H.S. (2023)
'Effect of adding propolis extract to sorghum grain varieties on in vitro gas parameters'
Journal of Elementology, 28(2), 485-496,
available: http://dx.doi.org/10.5601/jelem.2023.28.1.2356

RECEIVED: 28 December 2022
ACCEPTED: 4 June 2023

ORIGINAL PAPER

Effect of adding propolis extract to sorghum grain varieties on *in vitro* gas parameters*

Levend Coşkuntuna, Kadir Erten, Hasan Ersin Şamlı

Department of Animal Science
University of Tekirdağ Namık Kemal, Turkey

Abstract

In this study, the effect of adding propolis extract to sorghum grain varieties containing different levels of tannin on the *in vitro* gas parameters was investigated. The *in vitro* gas production, methane production, metabolic energy, net energy lactation and organic matter digestibility values were measured after 24, 48, 72, and 96 hours of incubation of the control group (without adding any additives to the sorghum varieties) and the group with 0.05% propolis extract in ethanol added to the sorghum varieties. The *in vitro* gas values formed at 24 hours in the Es8z102, Albanus, Sugar Drip, Gül Şeker, and Csr9303 sorghum varieties were found to be 164.5, 164.5, 158.6, 130.9, and 172.3, respectively. These values were determined to be 200.2, 201.4, 194.8, 176.6, and 198.4, respectively, with the addition of propolis. The highest *in vitro* gas production was found in the Csr9303 variety, which had the lowest tannin content. The *in vitro* methane values formed at 24 hours in the Es8z102, Albanus, Sugar Drip, Gül Şeker, and Csr9303 groups were found to be 26.12, 48.08, 32.47, 23.50 and 47.82, respectively. These values were found to be 26.69, 35.13, 30.39, 7.29, and 5.50, respectively, with the addition of propolis. With the addition of propolis, the gas production increased and the methane production decreased in variety Csr9303. The highest methane production was found in the Albanus variety. With the addition of propolis, methane production in the Albanus variety decreased, and it exhibited the highest gas production among the groups. Sorghum varieties with high tannin content had lower gas and methane production. With the addition of propolis, the amount of gas production increased and the amount of methane production decreased in groups with high tannin content. The addition of propolis extract to feeds containing 20 g kg^{-1} of tannin appears to have a positive effect on rumen digestibility and methane production.

Keywords: sorghum, propolis, *in vitro* gas production, *in vitro* methane production

Levend Coskuntuna, Assoc. Prof., Department of Animal Science, University of Tekirdag Namık Kemal, Tekirdag/Turkey, e-mail: lcoskuntuna@nku.edu.tr

INTRODUCTION

Sorghum (*Sorghum bicolor*) is the fifth most important cereal in the world and is used as an alternative to corn in feed. Its nutritional composition is similar to that of corn but it is more resistant to drought. It can be cultivated in poor soils and it can be used as an alternative to corn in crop rotations. It contains phytates, kaffir, and varying levels of condensed tannins (Puntigam et al. 2021). The interaction between tannins and sorghum proteins reduces both protein and starch digestibility. The protein barrier surrounding the starch granule is another limiting factor that can decrease the hydrolysis of sorghum grain starch by amylolytic enzymes (Parnian et al. 2014). It is also assumed that the presence of condensed tannins and other phenolic compounds in some varieties limits the range of nutrients. Especially, after tannins decompose due to low pH while passing through the abomasum, they bind to foods for intestinal digestion and reduce rumen microbial fermentation. However, it is assumed that this may also have positive effects, including the potential to increase the efficiency of energy use by preventing bloating and reducing methanogenesis (Amanzougarene et al. 2018). It has been reported that the tannin content of 1–4 % dry matter (DM) in feed reduces the breakdown of proteins in the rumen and causes rapid transition of chyme to the abomasum, while the tannin content of 5–11% DM has a negative effect on the feed consumption of animals (Budag 2009). The presence of condensed tannin at levels higher than 55 g kg^{-1} adversely affects the digestibility of feeds and feed consumption (Dschaak et al. 2011). Methods such as drying, soaking, fermentation, alkali treatment, and adding additives are used to reduce the tannin levels in feed (Alam et al. 2005, Wahyono et al. 2019).

Propolis is an organic substance with high antioxidant, antibacterial, and antifungal effects, which honeybees (*Apis mellifera*) collect from plants by adding resinous substances, their enzymes, and beeswax (Kutluca et al. 2007). Propolis with its high phenolic content can be used in animal nutrition. Many studies on the effects of propolis have been reported in the literature. In one study, it was observed that consumption of propolis at different levels in poultry had positive effects on performance and immunity of the birds (Galal et al. 2008; Seven and Seven 2008; Shalmany and Shivazad 2006). In ruminant animals, it has been reported that propolis reduces ammonia production in the rumen and improves nitrogen use (Oeztuerk et al. 2010). Positive results were obtained in live weight and daily weight gain by adding propolis extract to the rations of beef cattle (Zawadzki et al. 2011).

The present study aimed to determine the effect of adding propolis extract to sorghum varieties containing different levels of tannin on *in vitro* gas parameters.

MATERIALS AND METHOD

Materials

The plant material of the study consisted of sorghum varieties Es8z102, Albanus, Sugar Drip, Gül Şeker, and Csr9303 with different tannin and nutrient contents, which were obtained from the market. Propolis was purchased from a private company and extracted with ethanol. In the study, two treatment groups (1: control; 2: propolis extract addition) were formed.

Chemical analyses

Dry matter (DM), ash (A), crude protein (CP), and ether extract (EE) analyses of sorghum cultivars were performed according to the AOAC method (1990). The content of crude fiber (CF), which constitutes the cell wall's structure, was determined according to the method reported by Van Soest et al. (1991). The total tannin (TT) content of the sorghum cultivars was determined with the *n*-butanol method (Porter et al. 1985).

Determination of *in vitro* gas production parameters

The *in vitro* gas production technique was adopted. Samples (200 mg) of sorghum varieties without additives and sorghum varieties with 0.05% propolis extract added were placed in 100 ml glass injectors. The rumen fluid was taken from adult cattle as soon as they were slaughtered, and then transported to the laboratory at a constant temperature (38–40°C). The rumen fluid and the prepared saliva solution were mixed in carbon dioxide (1/2) and transferred to glass injectors. The injectors were left to incubate for 24, 48, 72, and 96 hours in a dark environment in an incubation cabinet set at 39°C. Methane production was determined by measuring the amount of gas formed in the glass injector every 24 hours. The metabolic energy, net energy lactation and organic matter digestibility values were calculated from the following formula (Menke and Steingass, 1987):

$$\mathrm{ME\ (MJ\ kg^{-1}\ DM)} = 0.157 \times \mathrm{GP} + 0.0084 \times \mathrm{CP} + 0.022 \times \mathrm{EE} - 0.0081 \times \mathrm{A} + 1.06$$

$$\mathrm{NE_L\ (MJ\ kg^{-1}\ DM)} = 0.115 \times \mathrm{GP} + 0.0054 \times \mathrm{CP} + 0.014 \times \mathrm{EE} - 0.0054 \times \mathrm{A} - 0.36$$

$$\mathrm{OMD\ (g\ kg^{-1}\ DM)} = 0.9991 \times \mathrm{GP} + 0.0595 \times \mathrm{CP} + 0.0181 \times \mathrm{A} + 9$$

GP: Gas production in 24 hours (ml); CP: crude protein (g kg^{-1} DM); EE: ether extract (g kg^{-1} DM); A: ash (g kg^{-1} DM).

The study was planned according to a 2 x 5 x 3 trial design. Statistical analysis of the data obtained was performed in an SPSS 22 package program. In the statistical evaluation of the data, one-way analysis of variance was used to determine differences between the groups, and the Duncan's multiple comparison test was used to compare group effects. The Pearson correlation analysis test was used to determine the relationship between parameters (Efe et al. 2000). The following modeling was used in the statistical evaluation of the research data.

$$Y_{ijk} = \mu + S_i + K_j + (SK)_{ij} + e_{ijk}$$

Yijk: i. supplement, j. observation value by variety

μ: population mean

S_i: i. effect of propolis

$K_{.J}$: j. effect of variety

$(SK)_{ij}$: supplement x Variety

e_{ijk}: error

RESULTS AND DISCUSSION

Raw nutrient content of sorghum grain varieties

The analysis results regarding the raw nutrient content of the grain sorghum varieties are presented in Table 1.

Table 1

Nutrients of sorghum varieties (g kg^{-1} DM)

Variety	DM	A	CP	EE	CF	NDF	ADF	TT
Es8z102	931.7^{a}	20.20^{bc}	112.2^{e}	52.40^{d}	20.10^{b}	139.3^{c}	39.10^{d}	3.996^{c}
Albanus	929.9^{b}	21.60^{b}	129.2^{a}	63.80^{b}	27.70^{b}	138.6^{c}	72.80^{b}	3.792^{d}
Sugar Drip	923.4^{d}	19.00^{c}	122.4^{b}	74.40^{a}	14.90^{c}	205.0^{b}	62.40^{c}	21.52^{a}
Gül Şeker	925.1^{d}	35.80^{a}	113.4^{d}	54.40^{c}	71.00^{a}	289.5^{a}	207.10^{a}	18.48^{b}
Csr9303	926.9^{c}	21.60^{b}	120.4^{c}	63.90^{b}	14.80^{c}	125.0^{d}	32.60^{e}	0.648^{e}
SEM	0.104	0.207	0.167	0.211	0.713	1.647	1.707	2.853

DM: Dry matter, A: ash, CP: Crude protein, EE: Ether extract, CF:crude fiber, NDF: Neutral detergent insoluble fiber, ADF: Fiber insoluble in acid detergent, TT: Total tannin SEM: Standard error of the mean, $^{a-e}$: The difference between groups with different letter superscripts in the same column is statistically significant (P<0.001).

The DM content of the sorghum cultivars ranged between 923.40 and 931.70 g kg^{-1}, with the Sugar Drip cultivar having the lowest DM content, while the highest DM content was found in the Es8z102 cultivar; the difference between the cultivars was statistically significant (P<0.001). In the literature, the DM content in sorghum grain was reported to be in the range of 887–985 g kg^{-1} (Parnian et al. 2014, Aguerre et al. 2015, Amanzougarene et al. 2018, González García et al. 2018, Dey et al. 2022).

The A content of the sorghum cultivars in this study was determined to be 19.00–35.80 g kg^{-1}, with the lowest A level found in the Sugar Drip and Es8z102 cultivars, and the highest one observed in the Gül Şeker cultivar. The difference between the cultivars was statistically significant (P<0.001). Dey et al. (2022) reported A contents of 107.6–127.1 g kg^{-1} in different sorghum varieties.

The CP values of the sorghum grain varieties were determined to be between 112.20 and 129.20 g kg^{-1}. The lowest CP value was observed in the Es8z102 variety, while the highest CP value was determined in the Albanus cultivar. The difference between the cultivars was statistically significant ($P<0.001$). In studies reported in the literature, CP in grain sorghum was found to be between 51.2 and 113.0 g kg^{-1} (Parnian et al. 2014, Amanzougarene et al. 2018, González García et al. 2018, Dey et al. 2022).

The CF values of sorghum grain cultivars were found to be between 14.80 and 71.00 g kg^{-1}. The lowest CF value was seen in the Csr9303 variety, the highest was found in the Gül Şeker variety, and the difference between the varieties was statistically significant ($P<0.001$).

The EE values for the sorghum cultivars varied between 52.40 and 74.40 g kg^{-1}. The Es8z102 cultivar had the lowest EE, while the highest was found in the Sugar Drip cultivar. The difference between the cultivars was statistically significant ($P<0.001$). In the literature, the EE value in grain sorghum was reported to be 11.0–41.7.2 g kg^{-1} (Chaugool et al. 2013, Parnian et al. 2014, Amanzougarene et al. 2018, Wahyono et al. 2019).

The NDF values for our sorghum cultivars varied between 125.00 and 289.50 g kg^{-1}, with the lowest value found in the Csr9303 cultivar and the highest one in the Gül Şeker cultivar. While there was no statistical difference between the Es8z102 and Albanus cultivars, the difference between the other cultivars was statistically significant ($P<0.001$).

The ADF values for our sorghum cultivars varied between 32.60 and 207.10 g kg^{-1}; the lowest value was seen in the Csr9303 cultivar, while the Gül Şeker cultivar had the highest level. The difference between the cultivars was statistically significant ($P<0.001$).

It was determined that the total tannin (TT) values in the tested grain sorghum cultivars ranged from 0.648 to 21.521 g kg^{-1}. Among the cultivars, the lowest tannin content was found in Csr9303, while the highest was found in the Sugar Drip cultivar. The difference between cultivars was statistically significant ($P<0.001$). Amanzougarene et al. (2018) reported TT value of different sorghum cultivars in the range of 0.2–8.1 g kg^{-1}.

In vitro gas production parameters of sorghum varieties

The results of the *in vitro* gas production analysis for the control and propolis extract groups are given in Table 2, Figure 1 and Figure 2.

It was determined that there were differences in terms of the *in vitro* gas production parameters. After 24 hours of incubation, the highest gas production was observed in the Csr9303 variety in the control group, while the lowest gas production was observed in the Gül Şeker variety and the difference was found to be statistically significant ($P<0.001$). Twenty-four-hour and forty-eight-hour incubation produced similar results. After 72 hours of incubation, there was no difference between the Es8z102, Albanus, and Sugar Drip cultivars in terms of gas production, but after 96 hours of incu-

Table 2

In vitro gas production parameters of sorghum varieties

Supplement	Variety	Gas production (hours)				Methane production						
		24	48	72	96	24	48	72	96	ME	NE_L	OMD
	Propolis	99.33	102.3	105.0	106.3	1.80	2.17	2.21	2.21	-	-	-
Control	Es8z102	164.5^{d}	167.5^{e}	169.4^{d}	173.8^{c}	26.12^{bc}	28.62^{b}	28.95^{b}	29.44^{b}	27.07^{d}	18.67^{d}	174.0^{d}
	Albanus	164.5^{d}	167.5^{e}	169.1^{d}	171.1^{c}	48.08^{a}	50.72^{a}	50.77^{a}	51.30^{a}	27.11^{d}	18.70^{d}	174.1^{d}
	Sugar Drip	158.6^{d}	162.0^{e}	164.0^{d}	164.3^{d}	32.47^{bc}	35.05^{b}	35.22^{b}	35.37^{b}	26.21^{d}	18.04^{d}	168.3^{d}
	Gül Şeker	130.9^{e}	132.2^{f}	134.2^{e}	136.2^{e}	23.50^{c}	26.02^{b}	26.17^{b}	26.57^{b}	21.79^{e}	14.81^{e}	140.5^{e}
	Csr9303	172.3^{c}	174.3^{d}	177.0^{c}	180.3^{bc}	47.82^{a}	50.39^{a}	50.82^{a}	51.32^{a}	28.33^{c}	19.59^{c}	181.9^{c}
Propolis	Es8z102	200.2^{ab}	207.9^{a}	210.5^{a}	211.8^{a}	26.69^{bc}	26.90^{b}	26.97^{b}	26.97^{b}	32.68^{ab}	22.78^{ab}	209.7^{ab}
	Albanus	201.4^{a}	208.4^{a}	211.1^{a}	212.4^{a}	35.13^{b}	35.32^{b}	35.52^{b}	35.52^{b}	32.91^{a}	22.95^{a}	211.1^{a}
	Sugar Drip	194.8^{b}	201.8^{b}	204.4^{a}	205.8^{a}	30.39^{bc}	30.60^{b}	30.60^{b}	30.60^{b}	31.88^{b}	22.19^{b}	204.4^{b}
	Gül Şeker	176.6^{c}	181.0^{c}	183.6^{b}	185.0^{b}	7.29^{d}	7.34^{c}	7.40^{c}	7.40^{c}	28.97^{c}	20.06^{c}	186.2^{c}
	Csr9303	198.4^{ab}	207.4^{ab}	210.1^{a}	211.4^{a}	5.50^{d}	5.69^{c}	5.69^{c}	5.69^{c}	32.43^{ab}	22.59^{ab}	208.0^{ab}
	SEM	4.058	4.482	4.541	4.491	2.644	2.771	2.781	2.808	0.638	0.467	4.055
P												
	Supplement	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Variety	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	S*V	0.002	0.013	0.02	0.016	0.000	0.000	0.000	0.000	0.002	0.002	0.002

ME: Metabolic energy (MJ kg^{-1} DM), NE_L: Net energy of lactation (MJ kg^{-1} DM), OMD: Organic matter digestibility (g kg^{-1} DM), SEM: The standard error of the mean, $^{a–f}$: The difference between groups containing different letters in the same column is statistically significant.

bation, gas production in the Es8z102 and Albanus cultivars increased in the control group and the increase was found to be statistically significant compared to the Sugar Drip cultivar ($p<0.001$). This was associated with the high tannin content of the cultivars suppressing gas production.

In the group of sorghum grains to which propolis was added, the highest gas production was observed in the Albanus, Es8z102 and Csr9303 varieties, while the lowest gas production was observed in the Gül Şeker variety after 24 hours of incubation. The difference was found to be statistically significant ($P<0.001$). While there was a difference between the Albanus and Sugar Drip cultivars in terms of gas production after 24 and 48 hours of incubation ($p<0.001$), there was no statistically significant difference between them in terms of gas production after 72 and 96 of incubation ($p>0.005$). At the end of the 96-hour incubation period, the differences in *in vitro* gas production values between sorghum cultivars with propolis added and the control group were found to be statistically significant ($p<0.001$).

It was determined that the addition of propolis to sorghum cultivars promoted gas production, and also increased the OMD values ($p<0.001$). It has been reported that tannins reduce the formation of methane in the rumen and prevent the breakdown of proteins (Kutlu and Freeer 2014). While the highest methane production in the control group was found in the Albanus and Csr9303 cultivars after 24 hours of incubation, the least

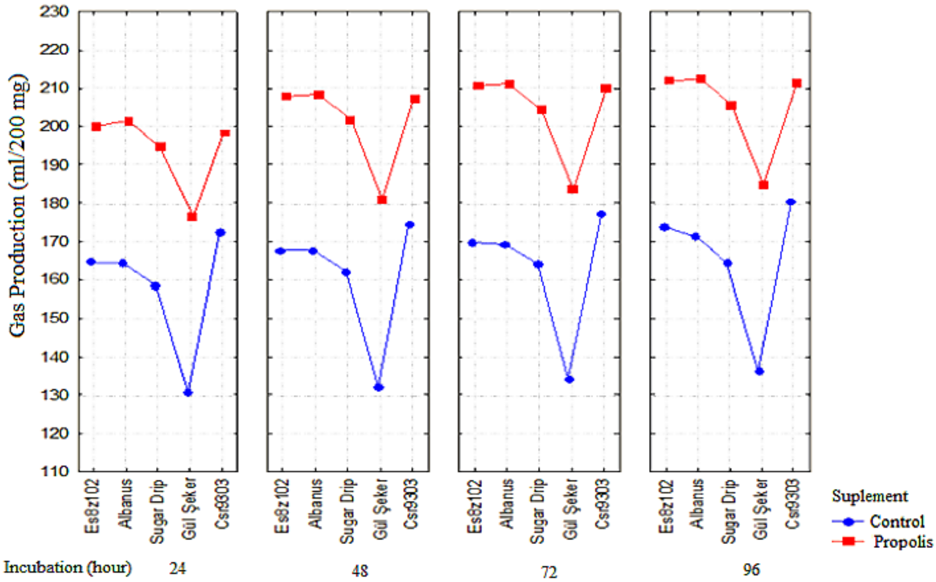

Fig. 1. *In vitro* gas production of sorghum cultivars

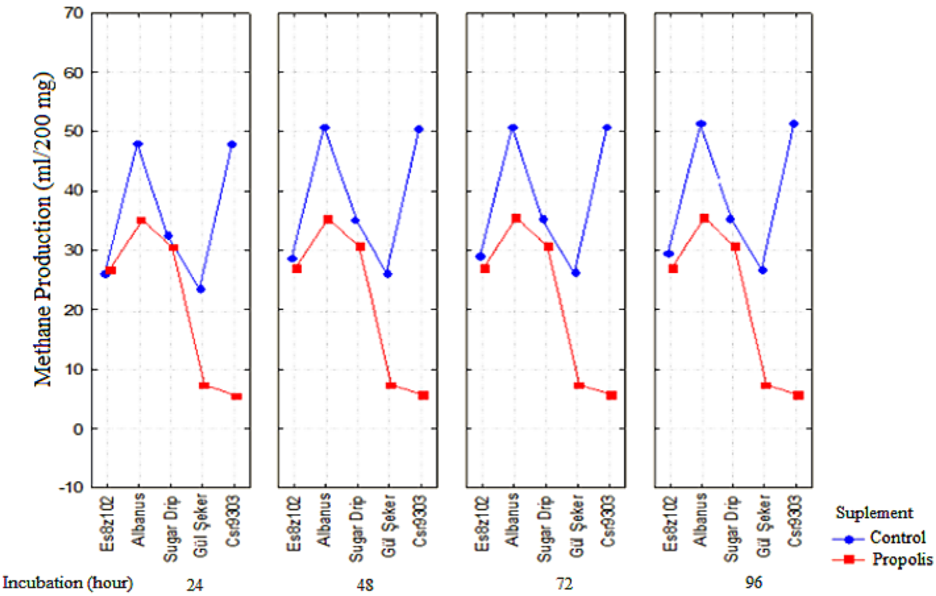

Fig. 2. *In vitro* methane production of sorghum cultivars

methane was produced in the Gül Şeker cultivar, and the difference was statistically significant (p<0.001). In the propolis group, the highest methane production was found in the Albanus variety, while the lowest one was observed in the Csr9303 variety. The difference between these cultivars was found to be statistically significant (p<0.001). This indicates that propolis reduced methane production rather than suppressing the amount of tannin. The Csr9303 variety is an example of this case. On the other hand, it was found that the addition of propolis had an effect on methane production in correlation with a certain amount of tannin in the plant. With the addition of propolis, methane production in the Gül Şeker and Albanus cultivars decreased statistically significantly compared to the control group (p<0.001), while there were no statistically significant differences in the Es8z102S and Sugar Drip cultivars.

The interaction between the added propolis and tannin can be linked to Csr9303, the variety with the lowest tannin content. The highest OMD value among the cultivars in the control group was determined for var. Csr9303, and the difference relative to the other cultivars was statistically significant (p<0.001), but there were no statistically significant differences between the Csr9303 cultivar and other cultivars (except Gül Şeker) in the propolis-added group. Propolis was suppressive towards methane and decreased the methane production in the Csr9303 variety. In the Albanus variety, with a slightly higher tannin content, the addition of propolis decreased the methane production, but the highest increase was achieved by the OMD value. Propolis did not affect methane production in the Es8z102 variety, with a high tannin content, but it increased the OMD value compared to the Csr9303 variety. The addition of propolis to sorghum cultivars suppressed the amount of tannin and increased plants' digestibility, but did not have any positive effect on methane production. Although the addition of propolis decreased the methane production in the Gül Şeker cultivar, the increase in the OMD value was the second highest after the Albanus cultivar.

Tannins form complexes with molecules of many compounds, such as proteins, polysaccharides, nucleic acids, and minerals, owing to the high number of free hydroxyl groups they contain and, especially, as a result of hydrophobic and hydrogen interactions (Silanikove et al. 2001, Min and Hart 2003, Makkar 2003). Since the effect of tannin was suppressed by propolis, the nutrient breakdown in the cultivars increased and the OMD value increased. It is known that dietary carbohydrates are fermented by microorganisms in the rumen, and VFA, and CO_2 and CH_4 are formed as the main products of this fermentation (Mitsumori and Sun, 2008). Propolis not only eliminated the negative effects of tannins but also reduced methane production. The Sugar Drip cultivar displayed a weaker response to propolis due to its high tannin content, hence the methane production did not change and this cultivar exhibited the lowest increase in OMD value.

In a study conducted with three different sorghum varieties (CSV-27, SPV-2018, and CSH 22SS), *in vitro* gas production$_{24}$ and methane production$_{24}$ values were found to be 127.69, 183.84, and 113.76 ml g^{-1} DM, and 21.57, 27.66, and 15.61 ml g^{-1} DM, respectively (Dey et al. 2022). In another study, *in vitro* gas production$_{24}$ by sorghum was determined to be 132.8 (ml g^{-1}, DM) (Parnian and Taghizadeh 2009). In a study conducted with sorghum grains, the *in vitro* gas production$_{24}$ was found to be 237.00–257.00 ml g^{-1}, OM) (Amanzougarene et al. 2018).

In another study with sorghum grains, *in vitro* gas production$_{24}$ was found to be 152.9 ml g^{-1} DM, and *in vitro* dry matter digestibility$_{24}$ was found to be 318.0 mg g^{-1} DM (Parnian et al. 2014).

Correlation analysis of sorghum grain varieties

The results of the correlation analysis for the crude nutrients and *in vitro* gas production parameters are given in Table 3. There was a negative relationship between the *in vitro* gas production and tannin content. Gas produc-

Table 3

Correlation values between raw nutrients and *in vitro* gas production parameters

	Supplement	Variety	Methane	Gas	DM	A	CP	EE	CF	NDF	ADF	TT
Variety	Control	–										
	Propolis	–										
Methane	Control	-0.647**	–									
	Propolis	0.251	–									
Gas	Control	-0,593*	0.597*	–								
	Propolis	-0.472	0.548*	–								
DM	Control	-0.549*	0.134	0.438	–							
	Propolis	–	0.327	0.548*	–							
A	Control	0.207	-0.405	-0.889***	-0.302	–						
	Propolis	–	-0.596*	-0.881***	–	–						
CP	Control	-0.268	0.708**	0.433	-0.054	-0.439	–					
	Propolis	–	0.500	0.465	–	–	–					
EE	Control	0.208	0.399	0.334	-0.583*	-0.513	0.700**	–				
	Propolis	–	0.332	0.275	–	–	–	–				
CF	Control	0.290	-0.445	-0.922***	-0.232	0.979***	-0.391	-0.524*	–			
	Propolis	–	-0.425	-0.855***	–	–	–	–	–			
NDF	Control	0.713**	-0.593*	-0.954***	-0.643**	0.818***	-0.390	-0.128	0.834***	–		
	Propolis	–	-0.323	-0.907***	–	–	–	–	–	–		
ADF	Control	0.429	-0.458	-0.960***	-0.404	0.956***	-0.322	-0.354	0.979***	0.917***	–	
	Propolis	–	-0.381	-0.895***	–	–	–	–	–	–	–	
TT	Control	0.933***	-0.529*	-0.700**	-0.785***	0.387	-0.162	0.317	0.429	0.846***	0.590*	–
	Propolis	–	0.029	-0.639*	–	–	–	–	–	–	–	–

* p<.05, ** p<.01, *** p<.001 DM: Dry matter, A: Ash, CP: Crude protein, EE: Ether extract, CF: crude fiber, NDF: Neutral detergent insoluble fiber, ADF: Fiber insoluble in acid detergent, TT: Total; Variety: Tannin content sorted from high to low

tion decreased with the increase in the amount of tannin. However, with the addition of propolis, the relationship between tannin and gas production decreased and gas production increased. A negative relationship was found between the amount of tannin and methane production, but this relationship turned to show a positive direction in response to the addition of propolis. The positive relationship between CP and methane production decreased with the addition of propolis, which can be explained by the effect of propolis on reducing methane production.

A strong negative correlation was found between CF, NDF, and ADF and gas production, affecting gas production as a result of cell walls reducing nutrient breakdown. It was seen that propolis reduced both the negative effects of grain on digestion and the production of methane. It can be argued that while the addition of propolis had a positive relationship with gas production, it had a negative relationship with methane production.

CONCLUSIONS AND SUGGESTIONS

The *in vitro* digestibility of sorghum varieties differed according to their tannin content. A high level of tannins prevents the breakdown of foods and reduces digestion levels. Although higher amounts of tannin in a feed ration reduce the production of methane, excessive consumption can have negative consequences for animals. The effect of such anti-nutritional factors can be reduced by the use of various additives. In this study, adding propolis extract to sorghum cultivars increased the *in vitro* gas production and OMD values. It also had a suppressive and reducing effect on methane production. It has been determined that propolis, which has a high antioxidant content, reduces both the negative effects of grain on digestion and methane production. However, with the increase in the amount of tannin in sorghum varieties, the functional mechanism of propolis was observed to weaken. It can be argued that the addition of propolis extract to feeds with a total tannin level of 20 g kg^{-1} may have a positive effect on rumen digestibility and methane production.

Conflicts of interest: The authors declare no conflict of interest.

REFERENCES

Aguerre M., Cajarville C., Repetto, J.L. 2015. *Impact of water addition, germination, ensiling and their association on sorghum grain nutritive value.* Animal Feed Science and Technology, 205: 75-81.

Alam, M.R., Kabir, A.K.M.A., Amin, M.R., McNeill, D.M. 2005. *The effect of calcium hydroxide treatment on the nutritive and feeding value of Albizia procera for growing goats.* Animal feed science and technology, 122(1-2): 135-148.

Amanzougarene Z., Yuste S., de Vega A., Fondevila M. 2018. *Differences in nutritional characteristics of three varieties of sorghum grain determine their in vitro rumen fermentation.* Spanish Journal of Agricultural Research, 16(3): e0608-e0608.

AOAC. 1990. *Official Methods of Analysis Association of Agricultural* Chemists. Virginia: DC.

Budağ C. 2009. *Baklagil tane yemleri ve ruminant beslenmede kullanımı.* YYÜ. Fen Bilim Enst Derg, 14(2): 88-107.

Chaugool J., Kondo M., Kasuga S., Naito H., Goto M., Ehara H. 2013. *Nutritional evaluation and in vitro ruminal fermentation of Sorghum cultivars.* J. Food Agric. Environ, 11: 345-351.

Dey A., Paul S.S., Umakanth A.V., Bhat B.V., Lailer P.C., Dahiya S.S. 2022. *Exploring feeding potential of stovers from Novel Sorghum (Sorghum bicolor* L.*) cultivars by in vitro fermentation pattern, gas production, microbial abundance and ruminal enzyme production in Buffalo.* Indian Journal of Animal Research, 56(1): 51-57.

Dschaak C.M., Williams C.M., Holt M.S., Eun J.S., Young A.J., Min B.R. 2011. *Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows.* J Dairy Sci, 94(5): 2508-2519.

Efe E, Bek Y, Şahin M. (2000). *SPSS'te çözümleri ile istatistik yöntemler* II. Kahramanmaraş Sütçü İmam Üniversitesi Rektörlüğü Yayınları, Kahramanmaraş, s. 223.

Galal A., Abd El-Motaal A.M., Ahmed A.M.H., Zaki T.G. 2008. *Productive performance and immune response of laying hens as affected by dietary propolis supplementation.* International Journal of Poultry Science, 7(3): 272-278.

González García U.A., Corona L., Castrejon-Pineda F., Balcells J., Castelan Ortega O., Gonzalez-Ronquillo M. 2018. *A comparison of processed sorghum grain using different digestion techniques.* Journal of Applied Animal Research, 46(1): 1-9.

Kutlu H.R., Serbester U. 2014. *Ruminant Beslemede Son Gelişmeler.* Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 2(1): 18-37.

Kutluca S, Genç F, Korkmaz A. (2006) *Propolis.* Samsun Valiliği, Tarım İl Müdürlüğü, Baskı: Çiftçi Eğitimi ve Yayım Şube Müdürlüğü, Samsun, ss: 1-57: 2006.

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 Ruminant Research, (49): 241-256

Menke K.H., Steingass H. 1987. *Schätzung des energetischen Futterwerts aus der in vitro mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. II. Regressionsgleichungen.* Übers. Tierernährg, 15(1): 59-94.

Min B.R., Hart S.P. 2003. *Tannins for suppression of internal parasites*. J. Anim. Sci., 81(E. Suppl. 2): E102-E109

Mitsumori M., Sun W. 2008. *Control of rumen microbial fermentation for mitigating methane emissions from the rumen.* Asian-Aust. J. Anim. Sci., 21: 1 144-154.

Parnian F., Taghizadeh A. 2009. *Evaluation of microwave irradiation effects on nutritive value of broom sorghum grain using an in vitro gas production technique.* In Proceedings of the British Society of Animal Science (Vol. 2009, pp. 182-182). Cambridge University Press.

Parnian F., Taghizadeh A., Paya H., Nobari B.B. 2014. *In vitro fermentation response to alkaline treated sorghum grain.* Journal of BioScience & Biotechnology, 3(1).

Porter L.J., Hrstich L.N., Chan B.G. 1985. *The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin.* Phytochemistry, 25(1): 223-230.

Puntigam R., Slama J., Brugger D., Leitner K., Schedle K., Wetscherek-Seipelt G., Wetscherek, W. 2021. *Fermentation of whole grain sorghum (sorghum bicolor (L.) moench) with different dry matter concentrations: effect on the apparent total tract digestibility of energy, crude nutrients and minerals in growing pigs.* Animals, 11(5), 1199.

Seven P.T., Seven İ. 2008. *Effect of dietary Turkish propolis as alternative to antibiotic on performance and digestibility in broilers exposed to heat stress.* Journal of Applied Animal Research, 34(2): 193-196.

Shalmany S.K., Shivazad M. 2006. *The effect of diet propolis supplementation on Ross broiler chicks performance.* International Journal of poultry science, 5(1): 84-88.

Silanikove N., Perevoltsky A., Provenza F.D. 2001. *Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants*. Animal Feed Science and Technology, 91: 69-81

Van Soest P.V., Robertson J.B., Lewis B.A. 1991. *Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.* Journal of Dairy Science, 74(10): 3583-3597.

Zawadzki F., Prado I.N., Marques J.A., Zeoula L.M., Rotta P.P., Sestari B.B., Valero M.V., Rivaroli D.C. 2011. *Sodium monensin or propolis extract in the diets of feedlot-finished bulls: effects on animal performance and carcass characteristics*. Journal of Animal and Feed Sciences, 20(1): 16-25.