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Yem Besin Maddelerinin *In Vıtro* Gaz Üretim Parametreleri Arasındaki İlişki

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ÖZ

Bu çalışmanın amacı *in vitro* gaz üretim tekniği kullanılarak yapılan çalışmalarda yemlerin besin madde bileşimleri ile *in vitro* gaz, metan ve OMS değerleri arasındaki ilişkiyi incelemektir. Bu çalışmada üç temel yem grubu ele alınmıştır. Bu grupları; kaba yem, kesif yem ve toplam rasyon karışımı (TMR) yemler oluşturmaktadır. Toplam 80 adet materyalin kuru madde (KM), ham kül (HK), nötr deterjanda çözünmeyen lif (NDF), asit deterjanda çözünmeyen lif (ADF), ham protein (HP), ham yağ (HY), *in vitro* gaz üretimi, *in vitro* metan üretimi ve sindirilebilir organik madde (OMS) değerlerine meta-analiz uygulanmıştır. En yüksek gaz üretimi TMR grubunda, en yüksek metan üretimi ve OMS değerleri ise küspe yemlerinde tespit edilmiştir. En yüksek NDF ve ADF değerleri saman grubunda görülmüştür, bu durum samanlarda en düşük *in vitro* gaz üretimi, metan üretimi ve OMS değerlerine sahip olmasını sağlamıştır. Yemlerin besin maddeleri ve *in vitro* gaz üretim parametreleri arasındaki ilişki incelendiğinde, gaz üretimi, metan üretimi ve OMS değerleri, NDF ve ADF ile negatif yönde ilişkilendirilirken, HK ve HP ile pozitif yönde ilişkilendirilmiştir.

Anahtar kelimeler: *In vitro* gaz üretimi, metan, kaba yem, kesif yem, TMR, meta-analiz

The Relationship Between The *In Vıtro* Gas Production Parameters Of Feed Nutrients

ABSTRACT

The aim of this study is to examine the relationship between the nutrient composition of feeds and *in vitro* gas, methane, and OMD values in studies using the *in vitro* gas production technique. In this study, three basic feed groups were considered. These feed groups include roughage, concentrate, and total mixed rations (TMR). Dry matter (DM), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), ether extract (EE), *in vitro* gas production, in total 80 materials meta-analysis was applied to *in vitro* methane production and organic matter digestibility (OMD) values. The highest gas production was determined in the TMR group, and the highest methane production and OMD values were determined in the pulp. The highest NDF and ADF values were observed in the straw group, which led to the lowest *in vitro* gas production, methane production, and OMD values in straw. When the relationship between feed nutrients and *in vitro* gas production parameters was examined, gas production (GP), methane production (MP), and OMD values were negatively correlated with NDF and ADF, while positively correlated with ash and CP.

Key words: *In vitro* gas production, methane, roughage, concentrate, TMR, meta-analysis

INTRODUCTION

In ruminant feeding, different methods are used to determine the nutritional values of feeds. These; *in vivo*, *in vitro* and *in situ* are methods. Although the *in vivo* method gives the most reliable results because the studies are carried out on live animals, the disadvantages of this method are that it requires a lot of labor, is difficult to implement, is difficult to follow, consumes a large amount of feed per animal, takes a long time, and is expensive (Ørskov, 1994; Getachew et al., 1998). *In vivo* method is an alternative to *in vitro* methods. Different analyses were made by the researchers in the methods, and it was tried to obtain results close to the *in vivo* method. *In vitro* techniques generally rely on the measurement of either products or fermentation residues. The most common method among these techniques is a two-stage digestion technique and a gas production technique (Tilley and Terry, 1963; Menke et al., 1979; Menke and Steingass, 1988).

The *in vitro* gas production technique, microbial feed it is a method based on the measurement of CO_2 gas released as a result of fermentation. *In vitro* under these conditions, the production of CO_2 gas occurs either directly as a result of the fermentation of carbohydrates in the feed or as a result of the reaction of volatile fatty acids (VFA) resulting from the fermentation of carbohydrates with the buffer solution (Menke et al., 1979).

Kılıç and Sarıçiçek (2006) in their review, the factors affecting the results of *in vitro* gas production technique studies; nutrient content of feeds, species and variety differences of feeds, harvest time and growing season, treatments applied to feeds, sample amount and size, fermentable substrate ratio of feeds, effect of animal species, feeding of animals, properties of rumen fluid, rumen conditions, amount of rumen VFA, The characteristics of the buffer used and the atmospheric pressure difference, the time of measurement, the application of the correction factor, the air bubbles accumulated in the syringes, the use of different mathematical models and equations.

The production of CO_2 and H_2 gases from the fermentation of the nutrients consumed by ruminant animals into methane (CH_4) by methanogen bacteria is called methanogesis (methane formation) (Hegarty and Klieve, 1999; Görgülü et al., 2009). Ruminant animals use 2-12% of the gross energy they receive from feed as methane energy (Canbolat et al., 2011). The amount of methane formed in the rumen of an adult cattle is around 300 liters/day (Breves and Leonhard-Marek, 2000), this energy value reaches approximately 4000 kcal, which means 1/3 of the energy requirement of a cattle with a live weight of 550 kg constitutes (Aksoy et al ., 2000). Ruminant animals cannot benefit from the energy contained in methane gas, so they throw it into the atmosphere by releasing it from their bodies. Therefore, this situation leads to ecological problems as well as economically (Öztürk, 2008). The share of methane gas produced by ruminant animals in the world, around 80-115 million tons annually, in global warming is 23 times higher than CO_2 and constitutes 15-20% of human-induced methane production (IPCC, 2001).

Kaya et al., (2012) applications that can be done to reduce methane production, adding vegetable oils to the ration, changing the ratio of roughage to concentrated feed, using methane inhibitors, They listed it as adding feed additives to the ration (herbal extracts, probiotics, organic acids, adsorbents), and immunization.

In this study, it was aimed to examine the relationships between nutrient content and *in vitro* gas production parameters of ruminant feeds.

MATERIAL AND METHOD

Using Central's online scientific platforms, a literature search was conducted using Google Scholar, Science Direct, and PubMed. Feeds *in vitro* For studies on gas production parameters, '*in vitro* gas production', '*in vitro* methane production' were used as keywords and the year of scanning of the articles was '2017-2022'. A total of 6,182 results were found related to keywords, studies that did not include nutrients, *in vitro* gas and methane production were excluded from these results and research was conducted with 80 studies. In the study, nutrient analyzes of roughage, concentrate and total mixed rations (TMR) feeds and statistical analyzes of *in vitro* gas production parameters and the relationship between them were examined. As the nutrient content of feeds; Dry matter (DM), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and ether extract (EE) data were evaluated.

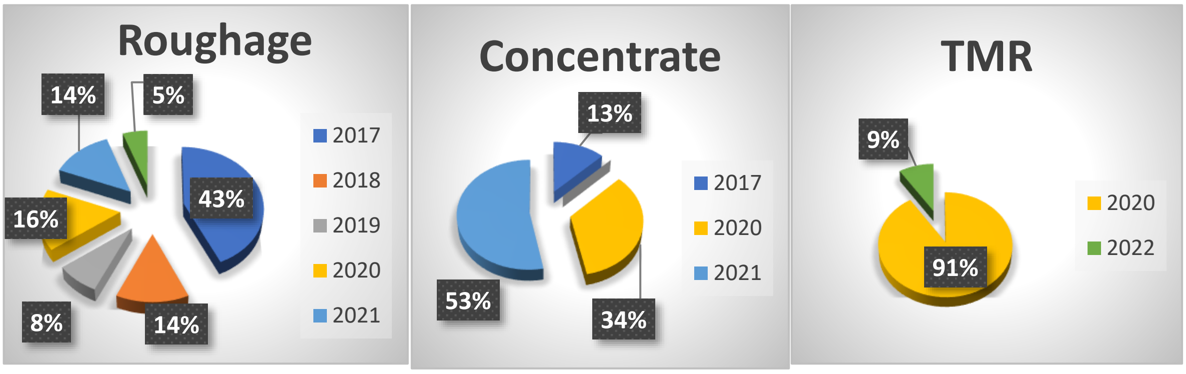
In the statistical evaluation of research data, one-way analysis of variance was used to determine the difference between groups, and Duncan's multiple comparison test was used to compare group effects. A Pearson correlation test was used to examine the relationship between groups (Efe et al., 2000).

FINDINGS and DISCUSSION

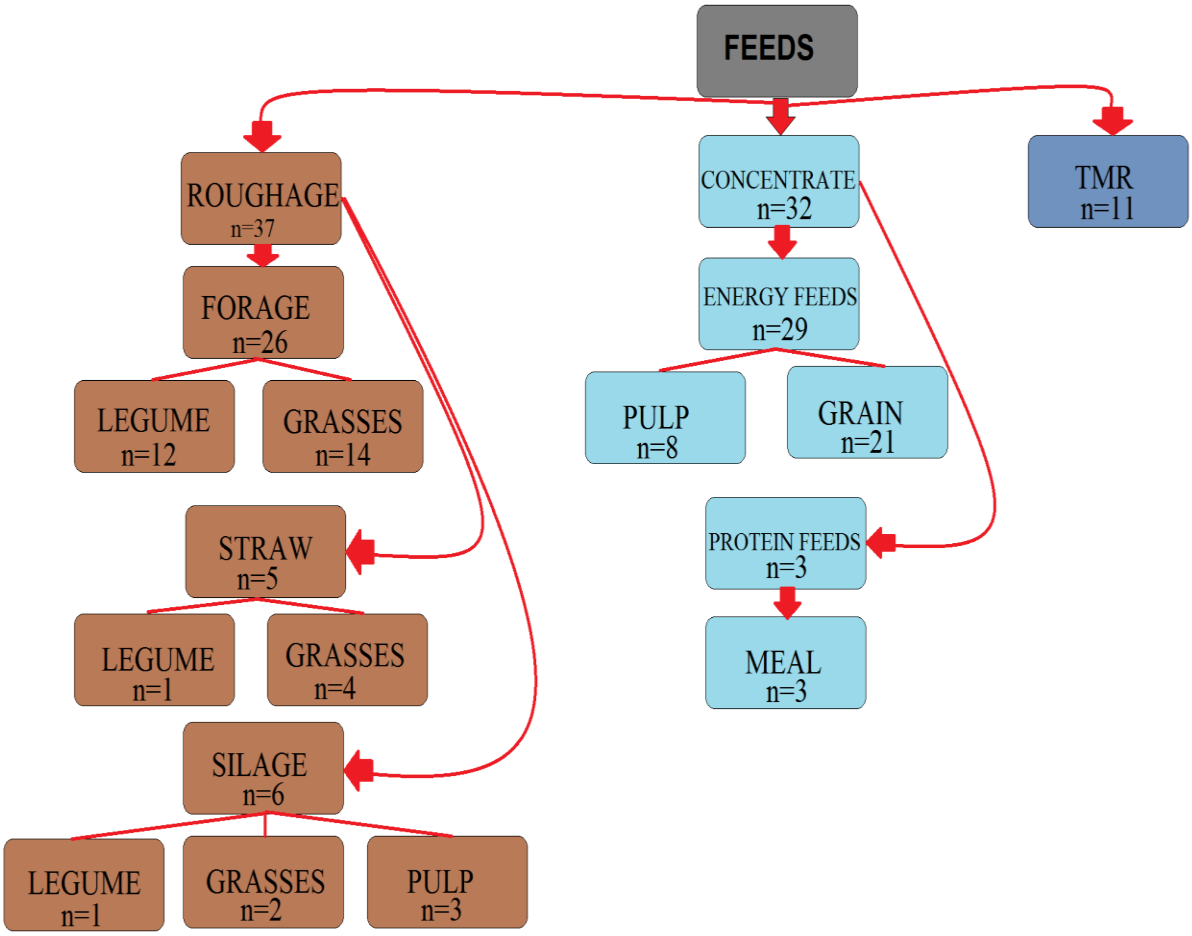
The grouping of the feeds used in the study is given in Graph 1 and Graph 2. Studies were carried out in three main groups: roughage, concentrate, and TMR. Roughage is classified into three types: hay, straw, and silage These are also listed as legumes, grasses, and pulp. Concentrated feeds were subdivided into energy feeds and protein feeds; energy feeds represented pulp and grain feeds, while protein feeds were pulp feeds. They were evaluated according to the results of studies conducted in the TMR group, regardless of the proportions of roughage and concentrate feed. 37 roughages, 32 concentrate feeds and 11 TMR feed were used for a total of 80 analyzed materials. The studies carried out and their proportional distribution by years are given in Table 1 and Graph 1. While the maximum number of studies was 27 in 2020, at least 3 were found in 2019 and 2022.

Table 1. Distribution of feeds by years in studies

Feed	2017	2018	2019	2020	2021	2022	Total
Roughages	16	5	3	6	5	2	37
Concentrate	4	-	-	11	17	-	32
TMR	-	-	-	10	-	1	11
Total	20	5	3	27	22	3	80



Graph 1. Distribution of the feeds used in the studies by years



Graph 2. Grouping of the feeds used in the studies

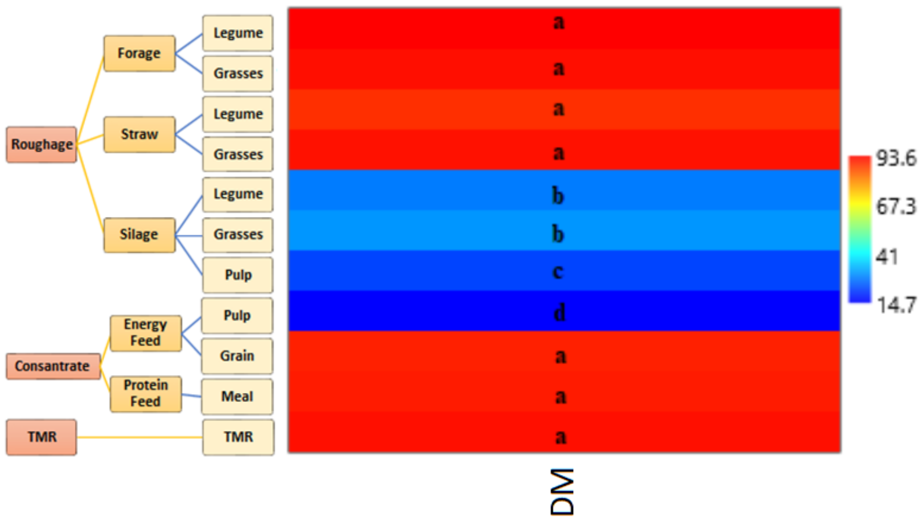
Table 2. Data from studies

Feed	Type	Kind	Year	Materiel	Source
Roughage	Hay	Legume	2017	*Desmodium*	Melesse et al ., 2017
			2017	*Medicago sativa*	Melesse et al ., 2017
			2017	*Vicia sativa*	Melesse et al ., 2017
			2018	*Onobrychis sativa*	Ulger et al ., 2018
			2018	*Onobrychis sativa*	Ulger et al ., 2018
			2018	*Onobrychis sativa*	Ulger et al ., 2018
			2019	*Medicago sativa*	Macit and Palangi 2019
			2019	*Vicia villosa*	Macit and Palangi 2019
			2019	*Trifolium repens*	Macit and Palangi 2019
			2020	*Medicago sativa*	Ozkan et al ., 2020
			2021	*Vicia sativa*	Ciftci and Gül, 2021
			2022	*Medicago sativa*	Selcuk et al ., 2022
		Grasses	2017	*Triticum aestivum*	Ayasan et al ., 2017
			2017	*Triticum aestivum*	Ayasan et al ., 2017
			2017	*Triticum aestivum*	Ayasan et al ., 2017
			2017	*Triticum aestivum*	Ayasan et al ., 2017
			2017	*Avena sativa*	Melesse et al ., 2017
			2017	*Lolium*	Melesse et al ., 2017
			2017	*Lolium*	Melesse et al ., 2017
			2017	*Lolium*	Melesse et al ., 2017
			2017	*Cynodon dactylon*	Melesse et al ., 2017
			2017	*Lolium*	Melesse et al ., 2017
			2017	*Lolium*	Melesse et al ., 2017
			2017	*Lolium*	Melesse et al ., 2017
			2017	*Miscanthus sp.*	Melesse et al ., 2017
			2021	*Hordeum vulgare*	Ciftci and Gül, 2021
	Straw	Legume	2018	Glycine max	Güleçyüz and Kılıç, 2018
		Grasses	2018	*Triticum aestivum*	Güleçyüz and Kılıç, 2018
			2020	*Triticum aestivum*	Ozkan et al ., 2020
			2021	*Triticum aestivum*	Ciftci and Gül, 2021
			2021	*Triticum aestivum*	Kılıç, 2021
	Silage	Legume	2021	Glycine max	Çiftçi et al ., 2021
		Grasses	2020	Zea mays	Ulger et al ., 2020
			2022	Zea mays	Dhakal et al ., 2022
		Pulp	2020	*Citrus lemon*	Ulger et al ., 2020
			2020	*Citrus sinensis*	Ulger et al ., 2020
			2020	*Citrus reticulata*	Ulger et al ., 2020
Concentrated	Energy Feed	Pulp	2017	*Citrus sinensis*	Ozkan et al ., 2017
			2017	*Citrus lemon*	Ozkan et al ., 2017
			2017	Citrus paradisi	Ozkan et al ., 2017
			2017	Citrus reticulata	Ozkan et al ., 2017
			2020	*Citrus aurantium*	Basar and Atalay, 2020
			2020	*Citrus aurantium*	Basar and Atalay, 2020
			2020	*Citrus aurantium*	Basar and Atalay, 2020
			2020	*Citrus aurantium*	Basar and Atalay, 2020
		Grain Feed	2020	*Avena sativa*	Ozkan et al ., 2020

			2020	*Triticum aestivum*	Ozkan et al ., 2020
			2020	*Zea mays*	Ozkan et al ., 2020
			2020	*Hordeum vulgare*	Ozkan et al ., 2020
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
			2021	*Avena sativa*	Kurt, 2021
	Protein Feed	Meal	2020	*Helianthus annuus*	Ozkan et al ., 2020
			2020	*Glycine max*	Ozkan et al ., 2020
			2020	*Gossypium hirsitum*	Ozkan et al ., 2020
TMR			2020	TMR	Purba et al ., 2020
			2020	TMR	Curzaynz-Leyva et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2020	TMR	Boga et al ., 2020
			2022	TMR	Kaya et al ., 2022

In the study, the analysis results regarding the nutrients and *in vitro* parameters of the feeds are given in Table 3, Table 4, and Table 5.

The DM values of the feeds in the studies are given in Graph 3. The DM values of the feeds varied between 14.74-93.60%. The highest DM values were observed in hay, straw, grain forages, protein feeds, and TMR groups, while the lowest was observed in the pulp and silage groups. No difference was found between legume-grass hay and straw (P>0.05). In the silage group, legume and grass silage showed similarity, while pulp silage had a lower DM value. The DM level in roughage was found to be statistically significant in hay and straw compared to the silage group (P<0.001). In the energy group, grain feeds had a higher DM value than the pulp, while protein feed showed similarity with the meal (P>0.05). The difference between the groups in concentrate feed was found to be statistically significant (P<0.001). TMR feeds were similar in terms of DM value to hay, straw, grain feed, and pulp.

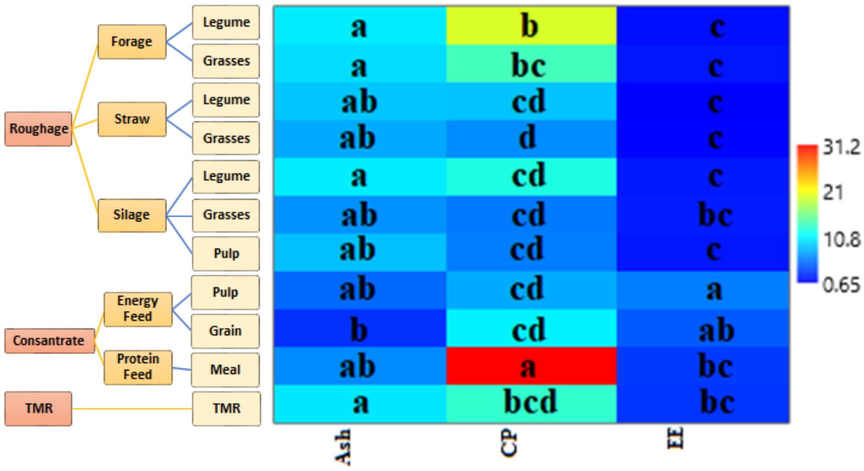


Graph 3. DM values of the feeds used in the studies

The CA, CP, and EE values of the feeds in the studies are given in Graph 4. The CA values of the feeds varied between 2.62-10.10%. While the highest CA values were seen in the hay, legume silage, and TMR groups, the lowest was seen in the grain feed group. There was no difference between the CA values of legumes and grass in the hay group (P>0.05). There was no difference between legume and grass feeds in the straw group, which has a lower CA value compared to dry grasses. In the silage group, the highest CA value was observed in legume silages, but there was no statistical difference between grasses and pulp silages (P>0.05). In energy feeds, the pulp had a higher CA value than the grain feed, while it was similar to the meal, and there was no statistical difference between them (P>0.05). The TMR group was found to have similar CA values with legume-grass hays and legume silages.

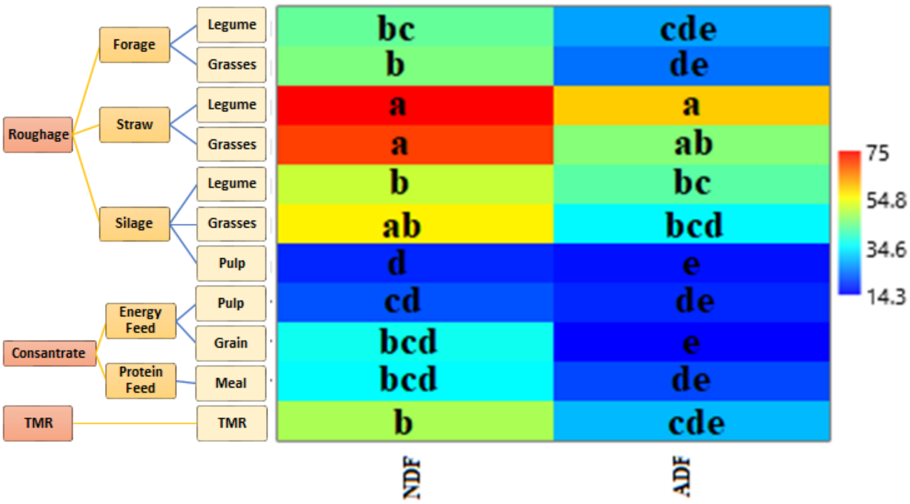
The CP values of the feeds varied between 4.92-31.20%. While the highest CP values were seen in the meal group, the lowest was seen in the grass straw. In the hay group, legume feeds were found to have higher CP values than grasses, but there was no statistical difference between them (P>0.05). In the straw group, legume feeds contain more CP than grasses, but there was no statistical difference between them. While the highest CP value was found in legume silages in the silage group, grasses and pulp silages showed similarity and no statistical difference was found between the groups (P>0.05). The highest CP value in roughage was determined in hay and statistically difference was found between groups (P<0.001). While grain feeds and pulps were similar in terms of CP value in the energy feed group, the meal had the highest CP value in the concentrate feed group and there was a statistical difference between the groups (P<0.001). While the TMR group had a statistically lower CP value than the meal group (P<0.001), there was no difference between the other groups.

The EE values of the feeds varied between 0.65-4.26%. While the highest EE values were seen in energy feeds, the lowest was seen in the straw group. In the hay group, the highest EE value was observed in grasses feeds, but there was no statistical difference between grasses and legumes (P>0.05). In the straw group, legumes and grass were similar in terms of EE value. In the silage group, the highest EE value was observed in the grasses silages, while the legume and pulp silages showed similarity and no statistical difference was found between the groups (P>0.05). The EE value in the roughage was similar and there was no statistical difference between the groups (P>0.05). In the energy feed group, the highest EE value was found in the pulp and there was no difference with grain feeds, while the protein meal had the lowest EE value in the concentrate feed group. The difference between the groups in concentrate feed was found to be statistically significant (P<0.001). The TMR group had similar EE values with grass silage and meal.



Graph 4. Ash, CP and EE values of the feeds used in the studies

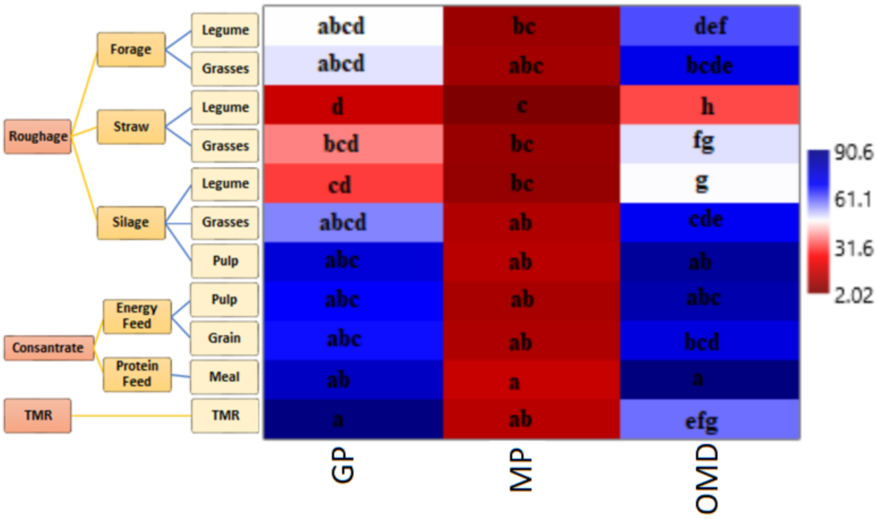
The NDF and ADF values of the feeds in the studies are given in Graph 5. The NDF values of the feeds varied between 17.32-75.02%. While the highest NDF values were seen in hay feed, the lowest were seen in the pulp silage group. Although the NDF value of the grasses and legumes was higher in the hay group, there was no statistical difference between them (P>0.05). In the straw group, legumes and grasses were similar to each other. In the silage group, the highest NDF value was determined in the grasses silage and there was no statistical difference between the legume silages, but it was found to be significant compared to the pulp silages with the lowest NDF value (P<0.001). While the highest NDF value in roughage was observed in the straw group, the difference between the groups was statistically significant (P<0.001). In the energy feed group, the grain feeds had higher NDF values than the pulp, which was similar to the meal and there was no statistical difference between the groups in the concentrate feeds (P>0.05). The TMR group, on the other hand, showed similarity to the legume hay and legume silage groups.



Graph 5. NDF and ADF values of the feeds used in the studies

The ADF values of the feeds varied between 14.33-58.84%. While the highest ADF value was seen in legume straw, the lowest was found in grain feeds. Although legume hays had higher ADF values than grasses, there was no statistical difference between them (P<0.05). The highest ADF value in the straw group is legumes, which were observed in the forage, but there was no statistical difference between them and the grasses straw (P>0.05). In the silage group, the highest ADF value was observed in legume silages, while there was no difference between them and grasses silages, it was found to be statistically significant compared to pulp silages (P<0.001). The highest ADF value in roughage was observed in the straw group, and a statistical difference was found between the groups (P<0.001). In the energy feed group, the pulp had a higher ADF value than the grain feed, and while it was similar to the meal, there was no statistical difference in the concentrate feed group (P>0.05). In terms of ADF value, the TMR group matched the closest legume hay. .

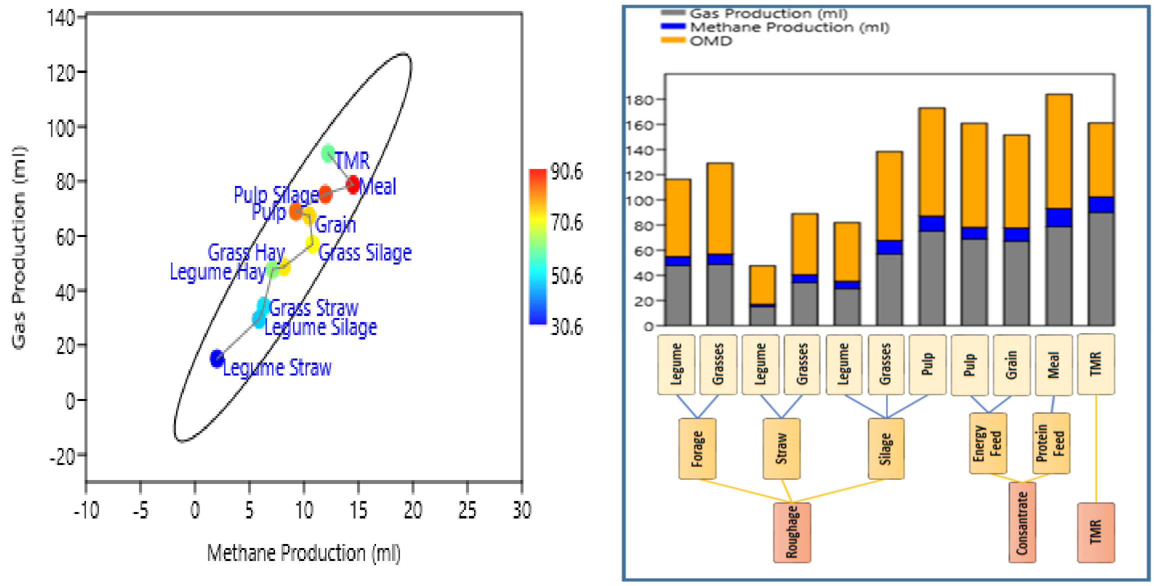
In vitro gas production parameters of feeds and the relationship between them are given in Graph 6 and Graph 7. *In vitro* gas production amounts of the feeds varied between 15.08-90.14 ml. The highest gas production was seen in the TMR group, while the lowest was seen in legume straw. Gas production amounts in hay feeds were similar in legume and grass feeds. Grain feeds produced more gas than legume feeds in the straw group. In the silage group, the highest gas production was observed in pulp silages, while the lowest was observed in legume silages. The highest gas production in roughage was seen in the silage group, and the lowest in the straw group, but the difference between the groups was not statistically significant (P>0.05). In the energy feed group, pulp and grain feeds showed similarities in the amount of gas production. Although the meals in the protein feed group provided more gas production than the energy feeds, there was no statistical difference between the groups in the concentrate feeds (P>0.05). The TMR group was found to be statistically significant in terms of gas production amount compared to the concentrate and forage groups (P<0.001).



Graph 6. *In vitro* gas production parameters of the feeds used in the studies

The *in vitro* methane production amount of the feeds varied between 2.02-14.50 ml. The highest methane production was seen in meal feeds, while the lowest was seen in legume straw. The highest methane production in hay was found in grasses, the highest methane production in the hay group was again seen in grasses. In the silage group, the highest methane production was observed in pulp and grasses silages, while the lowest was found in legume silages. While the highest methane production in roughage was observed in the silage group, there was no statistical difference between the groups (P>0.05). In the energy feed group, pulp and grain feeds had similar methane production, while meal had higher methane production. The protein feed group was found to be statistically significant compared to the energy feed group in terms of methane production amount in concentrate feeds (P<0.001). The TMR group was found to be statistically significant in terms of methane production compared to the concentrate and forage groups (P<0.001).

The OMD values of the feeds varied between 30.56-90.63 MJ/kg DM. The highest OMD values were observed in meals, while the lowest were observed in legume straw. In the hay group, grasses fodders had a higher OMD value than legume fodders, but there was no statistical difference (P>0.05). In the straw group, the grasses straws had a higher OMD value than the legume straw, and the difference between them was statistically significant (P<0.001). In the silage group, the highest OMD value was observed in the pulp silages, while the lowest was detected in the legume silages, and the difference between them was statistically significant (P<0.001). The highest OMD value in roughage was found in the silage group, and while it was statistically similar to the hay group, it was found to be significant compared to the straw groups (P<0.001). The highest OMD value of energy feeds was found in pulp feeds. While meals had the highest OMD value in the concentrate feed group, there was no statistical difference between protein and energy feeds (P<0.05). While the TMR group was similar to the roughage, it was found to have a lower OMD value than the concentrate feeds, and the difference between the groups was found to be statistically significant (P<0.001).



Graph 7. The relationship between *in vitro* gas productions parameters of the feeds used in the studies

Table 3. Nutrient (%DM) of feeds and *in vitro* studies gas analysis results for parameters

Feed	Type	Sort	DM	CA	NDF	ADF	CP	EE	GP_{24}	MP_{24}	OMD
Roughage	**Hay**	**Legume**	$93,6^{a}$	$10,0^{a}$	$42,34^{bc}$	$27,59^{cde}$	$19,95^{b}$	$1,19^{c}$	$46,33^{a-d}$	$6,82^{bc}$	$61,74^{d-f}$
		Grasses	$92,0^{a}$	$9,46^{a}$	$44,67^{b}$	$23,32^{de}$	$13,50^{bc}$	$1,56^{c}$	$48,75^{a-d}$	$8,15^{a-c}$	$72,23^{b-e}$
	Straw	**Legume**	$88,7^{a}$	$8,53^{ab}$	$75,02^{a}$	$58,84^{a}$	$8,41^{cd}$	$0,65^{c}$	$15,08^{d}$	$2,02^{c}$	$30,56^{h}$
		Grasses	$91,7^{a}$	$6,83^{ab}$	$74,79^{a}$	$46,06^{ab}$	$4,92^{d}$	$0,80^{c}$	$35,52^{b-d}$	$6,41^{bc}$	$49,00^{fg}$
	Silage	**Legume**	$27,4^{b}$	$10,10^{a}$	$50,42^{b}$	$41,7^{bc}$	$11,98^{cd}$	$1,67^{c}$	$29,47^{cd}$	$5,87^{bc}$	$46,58^{g}$
		Grasses	$30,2^{b}$	$6,5^{ab}$	$55,81^{ab}$	$34,14^{bcd}$	$5,51^{cd}$	$1,79^{bc}$	$56,93^{a-d}$	$10,70^{ab}$	$70,55^{c-e}$
		Pulp	$21,7^{c}$	$8,3^{ab}$	$17,32^{d}$	$15,68^{e}$	$5,67^{cd}$	$1,54^{c}$	$75,22^{a-c}$	$11,90^{ab}$	$85,75^{ab}$
Concentrate	**Energy**	**Pulp**	$14,7^{d}$	$4,79^{ab}$	$20,82^{cd}$	$17,41^{de}$	$7,49^{cd}$	$5,67^{a}$	$68,95^{a-c}$	$9,26^{ab}$	$82,65^{a-c}$
		Grain	$90,2^{a}$	$2,62^{b}$	$35,67^{bcd}$	$14,33^{e}$	$10,27^{cd}$	$4,26^{ab}$	$67,28^{a-c}$	$10,40^{ab}$	$73,87^{b-d}$
	Protein	**Meal**	$90,7^{a}$	$6,17^{ab}$	$34,36^{bcd}$	$20,03^{de}$	$31,20^{a}$	$2,96^{bc}$	$78,73^{ab}$	$14,50^{a}$	$90,63^{a}$
TMR			$92,0^{a}$	$9,88^{a}$	$48,09^{b}$	$28,99^{cde}$	$12,84^{bcd}$	$2,71^{bc}$	$90,14^{a}$	$12,22^{ab}$	$58,78^{e-g}$
SEM			3,780	0,478	1,885	1,398	0,774	0,226	3,230	0,428	1,560
P			0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000

[a - h] Differences between averages with different letters in the same column are significant. DM: Dry matter (%), NDF: Fiber insoluble in neutral solvents, ADF: Fiber insoluble in acid solvents, CP: Crude protein, EE: Ether extract, GP: Gas production (ml), MP: Methane production (ml), OMD: Digestible organic matter (MJ/Kg, DM), SEM: Standard error of the mean.

Table 4. *In vitro* gas production, methane production and OMD values of the feeds used in the *study*

Feed	GP_{24}	MP_{24}	OMD
Roughages	45.59^{c}	7.30^{c}	66.14^{b}
Concentrate	60.04^{b}	9.62^{b}	78.18^{a}
TMR	90.14^{a}	12.22^{a}	58.78^{b}
SEM	3,230	0.428	1,560
P	0,000	0,000	0,000

[a - c] Differences between averages with different letters in the same column are significant. GP: Gas production (ml), MP: Methane production (ml), OMD: Digestible organic matter (MJ/Kg, DM), SEM: Standard error of the mean.

Table 5. *In vitro* gas production, methane production and OMD values of the feeds used in the *study*

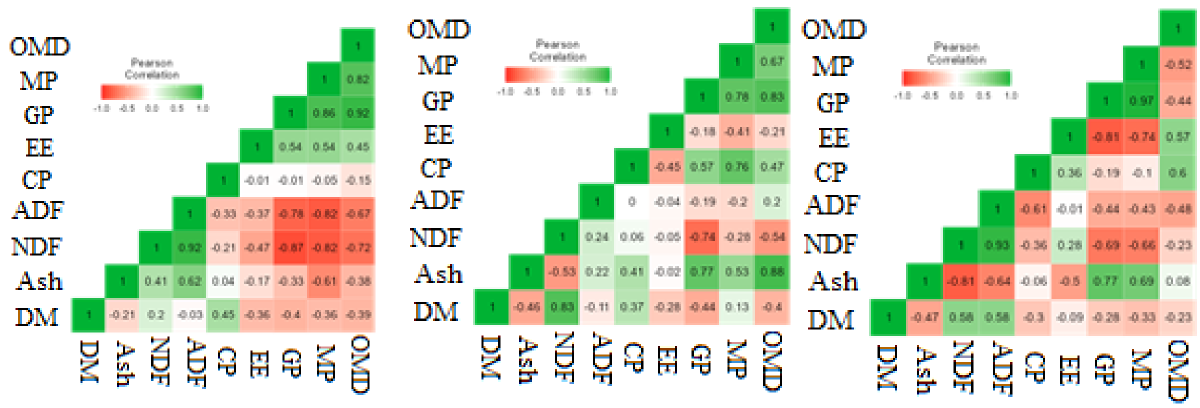
Feed	GP 24	MP 24	OMD
Hay	47.63^{b}	7.55^{c}	67.62^{bc}
Straw	44.73^{b}	7.15^{c}	50.34^{d}
Silage	61.50^{ab}	10.50^{bc}	74.15^{b}
Energy Feeds	66.98^{ab}	10.07^{bc}	76.78^{b}
Protein Feeds	78.73^{a}	14.50^{a}	90.63^{a}
TMR	90.14^{a}	12.22^{ab}	58.78^{cd}
SEM	3,230	0.428	1,560
P	0,000	0,000	0,000

$^{a-d}$ Differences between means with different letters in the same column are significant. GP: Gas production (ml), MP: Methane production (ml), OMD: Digestible organic matter (%), SEM: Standard error of the mean.

The results of the Pearson correlation test analysis regarding the nutrients and *in vitro* parameters of the feeds in the study are given in Graph 8.

Pearson's method was used on roughage. According to the correlation data, *in vitro* gas production, methane production, and OMD value were strongly negatively correlated with NDF and ADF, while a positive correlation was found with EE. While methane production has a strong negative relationship with DM, NDF, and ADF, it has a positive relationship with EE. Gas production, methane production and OMD were found to be strongly positively correlated. In concentrate feeds, gas production, methane production and OMD values were negatively correlated with NDF, while positive correlations were found with ash and CP. A strong positive relationship was found between gas production, methane production and OMD values. Gas production and methane production in TMR feeds were strongly negatively correlated with NDF, ADF and EE, but positively correlated with ash. While methane production was positively related to gas production, a negative correlation was found with OMD.

The relationship between *in vitro* gas production parameters and nutrients varies depending on the types of feeds. However, in general, gas production, methane production, and OMD values were negatively correlated with NDF and ADF, while positive correlations were found with CA and CP values.



Graph 8. Pearson feeds used in the study correlation analysis (roughage, concentrate and TMR)

CONCLUSION

Different methods used to determine the nutritional value of feeds are especially important for the nutrition of ruminant animals. Among these methods are *in vitro* methods, which are similar to *in vivo* methods but require less labor, have a practical application, are simple to use, require less feed and time, and have a larger study area. In this study, the results of studies using the *in vitro* gas production technique were discussed, and the relationships between the nutritional values of feeds and *in vitro* gas production and methane production were examined. Statistical analyses showed that *in vitro* gas production, methane production, and OMD values of feeds showed an inverse relationship with NDF and ADF, while a linear relationship was found with ash and CP values.

Conflict of Interest Statement: The authors of the article declare that there is no conflict of interest between them.

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