UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

https://doi.org/10.52973/rcfcv-e361781 Revista Científica, FCV-LUZ / Vol. XXXVI

Substitution of alfalfa hay with *Rumex pulcher* L. and its effect on in vitro gas production and ruminal fermentability

Sustitución de heno de alfalfa por *Rumex pulcher* L. y su efecto sobre la producción de gas in vitro y fermentación ruminal

***Mehtap Güney*[1]*, *Çağrı Kale*[2]**

[1] *Van Yuzuncu Yil University, Department of Animal Science, Faculty of Agriculture, 65090, Van, Türkiye*
[2] *Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, 65090, Van, Türkiye.*

Correspondence author: mguney@yyu.edu.tr

ABSTRACT

In this study, the effects of *Rumex pulcher* L. forage added to alfalfa hay as an alternative roughage source in ruminants at different mixing ratios (0, 25, 50 and 100%) on gas and methane production, in vitro digestibility properties and rumen fermentation parameters were determined by in vitro gas production technique. Experimental groups were formed of control (C: 100 % alfalfa hay), *Rumex pulcher* L. forage 1 (75 % alfalfa hay + 25 % *Rumex pulcher* L. forage), *Rumex pulcher* L. forage 2 (50 % alfalfa hay + 50 % *Rumex pulcher* L. forage) and *Rumex pulcher* L. forage 3 (100 % *Rumex pulcher* L. forage). Each experimental group was designed with 5 replicates. *Rumex pulcher* L. forage was significantly higher ($P < 0.001$) crude protein and lower neutral detergent fiber and acid detergent fiber content than alfalfa hay. While gas and methane production did not change in all treatments, *Rumex pulcher* L. forage 3 had the highest organic matter digestibility compared to the other treatment groups. As the rates of *Rumex pulcher* L. forage increased, an increase was observed in the amount of microbial protein synthesis parameters. Rumen parameters except acetic acid were different among all treatments. *Rumex pulcher* L. forage can be included in ruminant rations in order to reduce feed costs, due to its superior properties compared to alfalfa hay in terms of nutrient composition, *in vitro* digestibility and microbial protein synthesis. Furthermore, it is important to conduct further studies on this species, which grows uncultivated in nature, and explore it as an alternative forage source.

Keywords: Alternative roughage; in vitro gas and methane; rumen parameters

RESUMEN

En este estudio, se determinaron los efectos del forraje de *Rumex pulcher* L. añadido al heno de alfalfa como fuente alternativa de forraje en rumiantes, en diferentes proporciones de mezcla (0, 25, 50 y 100 %), sobre la producción de gas y metano, las propiedades de digestibilidad in vitro y los parámetros de fermentación ruminal, mediante la técnica de producción de gas in vitro. Se conformaron los siguientes grupos experimentales: control (C: 100 % heno de alfalfa), forraje de *Rumex pulcher* L. 1 (75 % heno de alfalfa + 25 % forraje de *Rumex pulcher* L.), forraje de *Rumex pulcher* L. 2 (50 % heno de alfalfa + 50 % forraje de *Rumex pulcher* L.) y forraje de *Rumex pulcher* L. 3 (100 % forraje de *Rumex pulcher* L.). Cada grupo experimental se diseñó con 5 repeticiones. El forraje de *Rumex pulcher* L. presentó un contenido significativamente mayor ($P < 0,001$) de proteína cruda y menor de fibra detergente neutra y fibra detergente ácida que el heno de alfalfa. Si bien la producción de gas y metano no varió en ninguno de los tratamientos, el forraje de *Rumex pulcher* L. 3 mostró la mayor digestibilidad de la materia orgánica en comparación con los demás grupos. A medida que aumentaron las tasas de forraje de *Rumex pulcher* L., se observó un incremento en los parámetros de síntesis de proteína microbiana. Los parámetros ruminales, a excepción del ácido acético, presentaron diferencias entre los tratamientos. La forraje de *Rumex pulcher* L. puede incorporarse a las raciones de rumiantes para reducir los costos de alimentación, debido a sus propiedades superiores en comparación con el heno de alfalfa en cuanto a composición nutricional, digestibilidad in vitro y síntesis de proteína microbiana. Además, es importante realizar estudios adicionales sobre esta especie, que crece de forma silvestre, y explorarla como fuente alternativa de forraje.

Palabras clave: Forraje alternativo; gas y metano in vitro; parámetros ruminales

Recibido: 19/09/2025 Aceptado: 06/12/2025 Publicado: 21/12/2025

CC BY NC SA

UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

INTRODUCTION

Today, problems such as decreasing agricultural land, climate change and environmental pollution have led to difficulties in the production and supply of feed resources for ruminants. It is inevitable that serious losses will occur because of the decrease in biodiversity due to these effects in animal and plant production. Studies examining the genetic characteristics of different species and creating conditions where they can better adapt to climatic conditions have become more important today [1].

In addition, new feed sources require more sustainable feed production. For this reason, non-traditional feed sources in ruminant nutrition have been increasing in recent years. Indeed, in livestock production areas, some naturally occurring and non-cultivated forages have the potential to partially or completely replace traditional forages in ruminant diets [2]. Therefore, these feeds, which can be an alternative for ruminants, can also be a solution to the roughage problem and contribute to the development of low-cost diets [3].

Rumex species, one of the alternative roughages for ruminants, have been used as medicine for humans from past to present, and studies on a wide variety of biological activities of this species have been reported. It has also been reported that some Rumex species are effective against many fungi and bacteria [4]. *Rumex acetosella* L., which is both commercially cultivated and collected from the wild in Türkiye, has been reported to have an average phenolic substance content and antioxidant capacity. However, it is stated that the bioavailability of these antioxidative properties is quite high [5].

In a study investigating the alternative forage properties of *Rumex acetosella* species, it was compared with alfalfa hay (AH) and sainfoin. The researchers reported that *Rumex acetosella* hay had a higher crude protein content and lower plant cell wall elements, and therefore had a higher digestibility value compared to the traditionally used alfalfa (*Medicago sativa*) hay and sainfoin (*Onobrychis viciifolia*) in animal nutrition. In addition, it was reported that the hay in question is good quality roughage and can also be used as protein feed due to its high protein content [6].

It has been reported that this herb grows naturally in many provinces from the east to the west of T*ürki*ye [6]. Researches stated that *Rumex pulcher* forage (RPH) has a strong antioxidant activity, all parts of this grass are hairy, its stems are square-shaped, the undersides of the nodes are not swollen, the leaf edges are toothed and it can grow upright between 10 and 60 cm [7].

Rumex pulcher L., a species belonging to the Polygonaceae family, has a high nutritional content and high degradation potential in the rumen, as well as having the effect of reducing greenhouse gas production in the rumen, and therefore it has been reported that this forage can be used as an alternative in ruminant nutrition [8]. Apart from the above study, no study was found examining the effects of RPH on ruminant animals. The fact that this forage, which grows freely in nature, can be used in ruminants may be a solution to some extent in terms of roughage production.

The objective of this study was to evaluate the substitution of AH by RPH on chemical composition, gas and methane production, *in vitro* digestibility and ruminal fermentation parameters.

MATERIALS AND METHODS

This study was conducted in accordance with the animal research procedures outlined in the institutional committee on animal use (case number 2024/10-10).

In this study, AH and RPH were obtained from Van province in Türkiye. The experimental groups were formed as control (C: 100 % AH), RPH1 (75 % AH + 25 % RPH), RPH2 (50 % AH + 50 % RPH) and RPH3 (100 % RPH) by supplementing increasing amounts of RPH on dry matter (DM) basis to AH, which is traditionally used in animal nutrition. Each experimental group was designed with 5 replications for rumen fermentation parameters and with 4 replications for microbial protein synthesis. A total of 45 (9 blank) *(Bos taurus)* gas production syringes (Model: Fortuna, 100 mL:1, 40 mm capillary tube, boro, amber grad, Poulten & Graf GmbH Wertheim/Germany) were used. In the study, AH and RPH were ground in a feed mill with a 1 mm sieve diameter (Retsch GmbH 5657 Haan, West- Germany) and then the mixing ratios were determined.

Rumen fluid was obtained from Simmental cattle (*Bos taurus*) of 600 ± 25 kg live weight, belonging to a farm fed a roughage based ration and brought to a slaughterhouse in Van. Rumen fluid was quickly brought to the laboratory in a thermos containing 39 ± 1 °C water, maintaining anaerobic conditions and filtered through a 4-layer cheese cloth. Then, it was mixed with artificial saliva in a ratio of 1/2 (rumen fluid/artificial saliva) under anaerobic conditions.

Chemical composition (DM, ash, CP, EE, ADF, NDF)

Dry matter (DM) and ash analyses of roughages and their mixtures were determined according to the Association of Official Analytical Chemists (methods 934.01 and 942.05, respectively) [9]. Crude protein (CP) analysis was determined by Kjeldahl steam distiller (method 984.13) [9], ether extract (EE) analysis was measured using an ANKOM XT15 device (Ankom®, Macedon, USA) [10], neutral detergent fiber (NDF and acid detergent fiber (ADF) analyses were analyzed using an ANKOM A220 device (Ankom, Macedon, USA) as described by Van Soest *et al.* [11].

İn vitro gas, methane production and digestibility assay

The study was conducted to determine the effect of AH and RPH mixtures on 24-h *in vitro* ruminal gas production [12]. The treatment groups (200 ± 1mg) were incubated with 30 mL of rumen fluid + artificial saliva mixture in 100 mL *in vitro* gas production syringes in a custom-made water bath at 39 ± 1 °C for 24 hours (h). For methane measurement, the gas formed in glass syringes was taken with a three-way plastic syringe system and injected into a computer-aided methane gas measuring device (Sensors Europe Analysentechnik GmbH, Erkrath, Germany) and the methane gas value (%) was read on the computer. By using 24-hours gas production (GP) and chemical composition parameters, metabolizable energy (ME) content and organic matter digestibility (OMD) of feed treatments were determined by the calculation [12].

$$OMD\ (\%) = 14.88 + 0.889GP + 0.45CP + 0.0651\ ash$$

$$ME\ (Mj\ /\ kg\ DM) = 2.20 + 0.136GP + 0.057\ CP$$

UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

In vitro microbial protein synthesis parameters

The treatment groups (500 ± 1 mg) were incubated with 40 mL of rumen fluid + artificial saliva mixture in 100 mL *in vitro gas* production syringes in a custom-made water-bath at 39 ± 1 °C for 24 h. At the end of the incubation, the gas was removed from the syringes and the material in the syringes for microbial protein analysis was transferred to 250 mL beakers, 70 mL of NDF solution was added and boiled for 1 h. After boiling, the material in the beakers was vacuum filtered through Gooch crucibles and dried in an oven (Nuve, FN 500, Turkey) at 105 ± 1 °C for 12 h. True digestibility (TD), microbial protein production (MP), microbial protein synthesis efficiency (MPSE) and partition factor (PF) were calculated using the formulas of Blummel *et al.* [13].

TDDM (mg) = incubated DM – remained DM,

TD (%) = (TDDM / incubated DM) X100

MP (mg /g DM) = TDDM – (GP X 2,2 mg/ml),

MPSE = ((TDDM – (2.2 x GP))/TDDM) X100

TF = TDDM/GP

Rumen fermentation parameters

In this study, pH measurement was made by taking the content transferred to 250 mL bottles in *in vitro* gas production syringes at the end of incubation with a digital pH meter (Thermo, Orion Star A11, USA; 0.01) immediately. Then, ammonia nitrogen analysis was performed in rumen fluids filtered with 4 layers of cheesecloth according to the Kjeldahl method. For this, rumen fluids were rotated at 4500 G for 5 min. 5 mL of rumen fluid was placed in glass tubes connected to a Kjeldahl distiller (Tecnal® TE 036/1) and water and sodium hydroxide were added, and the samples were distilled in a boric acid solution (30 g/L). The solutions obtained from the distillation (approximately 100 mL) were titrated with sulfuric acid (H_2SO_4; N/70) [14].

Rumen fluid samples were stored at -18 °C for VFAs (Acetic acid, Propionic acid, Butyric acid) by adding 2 mL of 1/1 HCl (absolute) to a 10 mL tube immediately after incubation [15]. After approximately 15 days, the samples were removed from the deep freezer (Arçelik, 2172 JEI, Türkiye) and thawed in the refrigerator overnight (Arçelik, 570431, Türkiye). Then, samples were centrifuged at 12000 G for 10 min. The supernatant obtained was sampled into 2 mL vials, and high performance liquid chromatography (HPLC, Thermo Scientific/Agilent, 1100, USA) conditions were adjusted with 300 x 7.8mm HPX-87H_ column as mobile phase 0.015 N H_2SO_4+0.0034 M EDTA, flow rate 0.7 mL/min, column temperature 45 ºC, detector PDA-DAD and 50 µL injection volume [16].

Statistical analysis

Statistical analyses of the study were performed using a linear model according to a completely randomized trial design, and differences at $P < 0.05$ were considered statistically significant. Differences between means were determined using the Duncan Multiple comparison test, and all analyses were performed in SAS 9.4 [17].

RESULTS AND DISCUSSION

The nutrient compositions of RPH and AH used in the study are in TABLE I. Differences between roughages were found to be significant in terms of all parameters analysed.

TABLE I
Chemical composition of Rumex pulcher L and Alfalfa hay %

	Rumex pulcher L	Alfalfa hay	P-value
Items	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	
DM	92.37 ± 0.05b	92.74 ± 0.03a	0.004
OM	79.57 ± 007b	85.77 ± 0.02a	< .0001
Ash	12.80 ± 0.02a	6.97 ± 0.01b	< .0001
CP	27.81 ± 0.19a	15.60 ± 0.17b	< .0001
EE	4.50 ± 0.14a	3.33 ± 0.04b	0.001
NDF	13.66 ± 0.27b	35.78 ± 0.55a	0.000
ADF	9.84 ± 0.03b	26.51 ± 0.11a	0.000

DM: Dry matter; OM: organic matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber

There are many naturally growing plant species in nature. These plant species play a very important role in the protection of natural diversity. In the literature searches, we did not find any studies other than one on the use of RPH in ruminants. Therefore, the results were compared with similar species of this forage.

The DM and OM content of RPH was lower than AH. The most striking thing was that the OM content of RPH was considerably higher than AH. This result was due to the higher ash content of RPH (12.80 %) compared to AH (6.97 %) (TABLE I). The sum of the mineral substances in the plant, and the mineral substances in the soil contaminated with the plant gives the ash content [18].

It is also stated that the difference in the ash content varies depending on the type of forage, fertilization and irrigation, soil structure, climate, harvest time, and the environmental conditions in which forage is grown [19]. In this study, it is thought that such high ash content is related to the plant species. In the study, the CP content of RPH (27.81 %) was determined to be higher than AH (15.60 %) (TABLE I). It is stated that RPH has CP levels similar to AH with high nitrogen fixation and that AH has the highest CP content among legume forage [20]. The study shows that the CP content of RPH is considerably higher than AH.

In addition, it is reported that the CP content in forages may vary depending on the species, vegetation, climate and nitrogen level of the soil [21]. The CP level obtained from RPH in the current study is similar to the values obtained from RPH [22]. Due to this very high CP content obtained from RPH, it is thought that it can be recommended for use in the nutrition of farm animals and can also be seen as a protein source feed material. It was observed that the NDF and ADF contents of RPH (13.66-9.84 %) were significantly lower than AH (35.78-26.51 %), respectively (TABLE I).

UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

It has been reported that ADF is an indicator of the digestibility of the plant and NDF is an indicator of the intake by animals, and that the ADF rate in feeds should be 30 % and below, and the NDF rate should be 40 % and below [23]. In the present study, the values obtained from the NDF and ADF results were found to be lower than the values obtained from RPH [6]. It is reported that the low fiber content of RPH may be related to the physiological state of the plant (early vegetation) [24].

İn vitro gas production, CH_4 production, OMD and ME values of RPH added to AH at different levels are in TABLE II. It was observed that adding RPH to AH at different rates did not change the total gas, methane values (%, mL), but changed the OMD and ME values, and the highest rate was obtained in the group using RPH3 (TABLE II).

TABLE II
Effect of using different rates of Rumex pulcher L. hay instead of alfalfa hay on in vitro gas production

		The ratio of experimental feeds				
	N	Control	RPH1	RPH2	RPH3	P-value
Items		$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	
Total gas*	5	146.16 ± 1.44	135.08 ± 0.98	136.62 ± 0.71	147.10 ± 0.37	0.162
Methane, %	5	11.66 ± 0.47	11.54 ± 0.25	11.53 ± 0.21	11.32 ± 0.27	0.895
Methane, mL	5	11.83 ± 0.56	11.95 ± 0.23	11.99 ± 0.28	11.74 ± 0.04	0.949
OMD, %	5	52.32±0.59b	50.72±0.34c	52.22±0.44b	56.54±0.33a	<.0001
ME**	5	7.97±0.20b	7.84±0.13b	8.12±0.10b	8.80±0.05a	0.001

OMD: Organic matter digestibility; ME: Metabolizable energy; [a,b]Different superscript letters in the same row represent significant difference, $\bar{x} \pm S_{\bar{x}}$: mean±standart error mean; *mL/g DM; ** Mj/kg DM

Methane and carbon dioxide gas, which are formed because of the fermentation of nutrients in ruminants, are one of the important sources of greenhouse gas emissions. It is also considered an important problem as the cause of climate change worldwide. Using RPH at different rates instead of AH did not change the *in vitro* gas production (mL/g DM), methane (%) and methane (mL) production (TABLE II). In a study comparing RPH with some weeds with forage potential, it was stated that *in vitro* gas production was lower, feed value was higher, and it could be used in ruminant animals due to its digestibility and greenhouse gas reducing effects [8].

A other study from various non-traditional plants, including Rumex forage, reported that forages with high CP and low fibre content had a reducing effect on methanogens and protozoa in the rumen in ruminants [25], thereby reducing greenhouse gas emissions [24 , 26]. It has been reported that methane production, as well as gas production, is related to the amount of fermentable carbohydrates [27].

In current study, it was expected that gas and methane production would decrease as the rates of RPH increased, but the results were not as expected. This situation showed that the use of RPH instead of AH did not inhibit ruminal microbial activity, continued and was a good nutrient source for rumen microorganisms, while it had no effect on methanogens in the rumen.

It stated that in addition to measuring gas production in the *in vitro* gas production technique, determining the degree of digestion allows more information to be obtained about the feeds [27]. In addition to it is important to determine energy and digestible nutrients, while determining the differences between feeds [28]. In the study, OMD and ME levels were observed to reach the highest value when 100 % RPH was used (TABLE II). The higher OMD and ME levels obtained when 100 % RP was used may be due to the lower plant cell wall structure of RPH compared to AH. Furthermore, low cell wall coverage is thought to be a result of hydrolysis into simple sugars, and the higher OMD of RPH compared to AH may be due to the solubility of its metabolizable energy. It was stated that oak nuts supplementation to AH increased OMD and ME levels *in vitro* [29].

The *in vitro* digestibility and ME values obtained from the study were found to be similar to the results obtained from *Rumex acetocella* [6 , 22].

In the study, the true digestion degree and microbial protein synthesis parameters are in TABLE III. When the table was examined, the difference between all treatments examined was significant. As the AH rates decreased, an increase was determined in MP, PF and TD, with the highest rates observed in RPH3.

Protein degradation in the rumen is the result of microbial activity, and varies depending on the type of proteins, ruminal dilution rate, ruminal pH, fermented substrate and dominant species of rumen flora [30]. Moreover, protein and carbohydrates in the composition of feeds affect microbial protein yield in rumen [31].

In the study, MP values increased as RPH rates increased. While the lowest *in vitro* MP amount was obtained from 100 % AH with 172.63 %, the highest value was reached in 100 % RPH with 242.48 %. The degree of true digestibility increased in accordance with the MP values. The highest value of 88.16 % was obtained from 100 % RPH (TABLE III). It is thought that the increase in protein degradation in the rumen due to the higher CP content of RPH compared to AH increases MP, and low NDF and ADF content increased TD values due to increased soluble carbohydrates in the rumen. With these properties, RPH can be considered as an alternative forage source.

UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

It is reported that the addition of 1 unit of oak nuts to the ration results in an increase of 0.6477 in microbial protein production, 0.4105 in microbial protein synthesis efficiency, and 0.262 in the partitioning factor [32]. In another study, it was stated that oak nuts (*Quercus* spp.) supplementation added to alfalfa hay at different levels reduced the amount of protein destroyed in the rumen and increased bypass proteins, *in vitro* organic matter digestibility and metabolizable energy values as a result of 24-h *in vitro* incubation [29]. It is reported that caramba hay has higher TD ratios than vetch (vicia) and alfalfa hay [21].

TABLE III
Effect of using different rates of Rumex pulcher L. hay instead of alfalfa hay on in vitro microbial proteins synthesis parameters and TD

		The ratio of experimental feeds				
	N	Control	RPH1	RPH2	RPH3	p
		$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	
MP	5	172.63 ± 3.33c	178.15 ± 4.08c	201.27 ± 10.88b	242.48 ± 4.61a	< .0001
MPSE	5	55.03 ± 0.93ab	53.06 ± 1.25b	54.16 ± 2.22b	59.00 ± 0.75a	0.044
PF	5	1.91 ± 0.02c	1.96 ± 0.02c	2.10 ± 0.06b	2.33 ± 0.03a	< .0001
TD, %	5	67.04 ± 0.18d	72.13 ± 0.41c	79.61 ± 1.01b	88.16 ± 0.88a	< .0001

MP: Microbial protein; MPSE: Microbial protein synthesis efficiency;; PF: Partitioning factor; TD: True digestibility;; [a,b,c]Different superscript letters in the same row represent significant difference, $\bar{x} \pm S_{\bar{x}}$: mean±standart error mean

It reported that using *Rumex asotocella* hay instead of AH in lamb diets decreased *in vitro* methane production while increasing MP, MPSE, PF and TD [22]. The researchers also stated that there was a negative relationship between methane production and microbial protein synthesis parameters. However, in the current study, while the amount of methane did not decrease, an increase in PF, MP, and TD was observed. These results revealed that rumen fermentation did not suppress methane-producing bacteria and hence fermentation.

Moreover, it can be said that microbial protein production increased positively with the increase in energy and protein in roughage. It is stated that the digestion rate and feed consumption of forages with high PF values are high [13]. It has also been reported that the PF values of feeds are the most important element in determining the microbial protein synthesis efficiency, and that feeds with high PF values also have high microbial protein synthesis efficiency [33]. In the study, RPH3 has the highest PF value, microbial protein production and TD are also high.

İn vitro rumen fermentation parameters of the roughage mixtures used in the study were determined and are in TABLE IV. Differences between treatment groups were significant for all parameters examined except AA concentration.

TABLE IV
Effect of using different rates of Rumex pulcher L. hay instead of alfalfa hay on rumen fermentation parameters

		The ratio of experimental feeds				
	N	Control	RPH1	RPH2	RPH3	P
Title		$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	
pH	5	6.35 ± 0.007d	6.41 ± 0.007c	6.45 ± 0.004b	6.52 ± 0.007a	< .0001
NH_3, mL/100	5	29.49 ± 0.38d	35.74 ± 0.21c	39.22 ± 0.56b	42.09 ± 0.63a	< .0001
AA, mmol/L	5	92.56 ± 1.13	90.13 ± 1.01	94.16 ± 2.64	95.43 ± 0.43	0.1261
PA, mmol/L	5	22.18 ± 0.61a	21.93 ± 0.24a	22.32 ± 0.56a	19.99 ± 0.49b	0.0142
BA, mmol/L	5	14.06 ± 0.60b	19.96 ± 1.10a	14.27 ± 0.49b	12.91 ± 0.20b	< .0001

NH_3: ammonia; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; [a,b,c,d] Values within a column with different superscripts differ significantly

The effect of using RPH on rumen pH values was statistically significant (TABLE IV). It is seen that rumen pH values increased as RPH rates increased. The highest pH value was reached in the experimental group where RPH3 was used. All pH values in the study varied between 6.35 and 6.52 and were found to be within acceptable limits for rumen fermentation [34]. It is reported that rumen pH did not change significantly with increases in protein, energy and rumen degradable protein levels and was determined in the range of 6.88-7.22 [35].

In this study, ammonia nitrogen levels also increased significantly in parallel with the increase in RPH rates. It is determined that increasing pH and ammonia rates are related to increased fermentation because of RPH degradation. In this study, ammonia nitrogen levels were considerably higher in RPH compared to AH. Therefore, while ammonia nitrogen levels were 29.49 mL/100 mL in control, also increased as RP ratio increased and reached 42.09 with RPH3. It is seen as a natural result that high ammonia nitrogen production in RPH supports MP production. Increased MP production has been reported to increase ammonia nitrogen utilization and efficiency of fibre

UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

digestion, thus ensuring optimum utilization of the diet offered to the animal [36].

In fact, in present study, OMD and TD was also the highest in the RPH treatment group, and it increased with the increase in RPH rate. It can be said that the degradation of RPH in the rumen is high, and the microbial activity occurring in the rumen degrades the protein source in RPH into ammonia. It is stated that a low rumen degradable protein level may reduce ruminal ammonia nitrogen levels, DM intake, and microbial protein synthesis [37].

Total VFA is a product of rumen microbial activity resulting from the digestion of the energy source in the feed [38]. It is stated that differences in rumen degradable protein levels may alter VFA production. In addition to the amount and composition of roughage in the diet affects the metabolism of cellulose degradation and VFA production [39]. In the study, *in vitro* rumen AA rates did not change with different rates of RPH contribution to AH. However, PA levels were higher in the treatment group with RPH1 than in the other groups. The reason for the higher PA in this treatment group is unknown, but it is thought that the synergistic effect of the roughage may have increased PA levels.

CONCLUSIONS AND IMPLICATIONS

In the treatment groups prepared from RPH mixtures at different rates instead of AH, 100% RPH provided a significant increase in in vitro digestibility, MP, and TD compared to AH and other mixtures. RPH grown freely in nature can be considered an optional roughage source due to its high digestibility, rich nutrient composition, crude protein content, and low cell wall elements. In today's world of drought, *Rumex pulcher* L. can be considered a highly valuable roughage source for sustainable animal husbandry. In addition, researching the productivity characteristics of this forage for cultivation is very important for ruminant nutrition, especially due to its high HP, low ADF and NDF content.

Conflicts of interest

The authors declare that there is no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Rojas-Downing MM, Nejadhashemi AP, Harrigan T, Woznicki SA. Climate change and livestock: Impacts, adaptation, and mitigation. Clim. Risk Manag. [Internet]. 2017; 16:145-163. doi: https://doi.org/gfvdh6

[2] Halmemies-Beauchet-Filleau A, Rinne M, Lamminen M, Mapato C, Ampapon T, Wanapat M, Vanhatalo A. Review: Alternative and novel feeds for ruminants: nutritive value, product quality and environmental aspects. Animal [Internet]. 2018; 12(2):295-309. doi: https://doi.org/gffvw6

[3] Wanapat M, Cherdrhong A, Phesatcha K, Kang S. Dietary sources and their effects on animal production and environmental sustainability. Anim. Nutr. [Internet]. 2015; 1(3):96-103. doi: https://doi.org/gfzm9h

[4] Kuruüzüm A, Demirezer Ö. Rumex türlerinin kullanılışı ve biyolojik aktiviteleri. FABAD J. Pharm. Sci. 1997 [cited 20 Jul 2025]; 22:21-26. Available in: https://goo.su/zWDbh7

[5] Sabuncu M, Konak M, Şahan Y. Rumex acetosella L'nin biyoalınabilir antioksidan özelliklerinin belirlenmesi. Bursa Uludag Üniv. Ziraat Fak. Derg. [Internet]. 2019 [cited 20 Jul 2025]; 33(2):197-207. Available in: https://goo.su/0G5mz7

[6] Güney M, Aydın R. Evelik otu'nun (*Rumex acetosella*) yem değeri ve *in vitro* gerçek sindirilebilirliğinin belirlenmesi. Yuzuncu Yil Üniv. Fen Bilimleri Enst. Derg. [Internet]. 2024; 29(2):780-786. doi: https://doi.org/qjgb

[7] Taşkın T. Çakırlı (Bursa-Orhangazi) yöresinde yenebilen bazı yabani bitkilerin antioksidan aktivitelerinin incelenmesi. [master thesis on Internet]. Türkiye: Marmara Üniversitesi, Sağlık BilimLeri Enstitüsü. 2011 [cited 20 Jul 2025]. 129 p. Available in: https://goo.su/UKGLOOg

[8] Barros-Rodriguez M, Rovalino-Nunez V, Núñez-Torres O, Mera-Andrade R, Artieda-Rojas J, Vaca-Freire L, Curay-Quispe S, Ortiz-Tirado P, Solorio-Sanchez J, Iraola J. Composición química, cinética de degradación ruminal y producción de gas *in vitro* de arvenses con potencial forrajero. *Livest. Res. Rural Dev. [Internet]. 2017 [cited 20 jul 2025]; 29(4):1-12. Available in:* https://goo.su/qnqFI

[9] Association of Official Analytical Chemistry (AOAC). Official Methods of Analysis. 18th ed. Washington, DC: AOAC International; 2010.

[10] Ankom. Crude fiber analysis in feeds by filter bag technique. Macedon, NY: ANKOM Technology; 2008 [cited 20 Jul 2025]. https://goo.su/GU922I

[11] Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. [Internet]. 1991; 74: 3583-3597.doi: https://doi.org/b6c78f

[12] Menke KH. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. [Internet]. 1988; 28:7-55. Available in: https://goo.su/6eMAIqN

[13] Blümmel M, Steingass H, Becker K. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and N-15 incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. [Internet]. 1997; 77(6): 911-921. doi: https://doi.org/dq2dpc

[14] Markham R. A steam distilation apparatus suitable for micro-kjeldahl analyses. J. Biochem. [Internet]. 1942; 36(10-12):790-791. doi: https://doi.org/qjgc

[15] Senyuz HH, Karsli MA. The substitution of corn silage with potato pulp silage at differing level in dairy cows on milk yield, composition and rumen volatile fatty acids. J. Fac. Vet. Med. Erciyes Univ. [Internet]. 2021; 18(1): 1-10. doi: https://doi.org/qjgd

[16] Castillos-Vargas JA, Costa de Araújo T, Mezzomo R. Extraction, identification, and quantification of volatile fatty acids (VFA) in rumen fluid samples using Reverse Phase High-Performance Liquid Chromatography with Diode Array Detector (RP HPLC-DAD). Res. Sq. [Internet]. 2020; 1:1-11. doi: https://doi.org/qjgf

UNIVERSIDAD DEL ZULIA
Serbiluz
Sistema de Servicios Bibliotecarios y de Información
Biblioteca Digital
Repositorio Académico

[17] Statistical Analysis System (SAS). SAS/STAT Software: Hangen and Enhanced, Version 9.4, Cary, North Carolina, USA: SAS Institute Inc. 2014.

[18] Weiss B. Feeding high ash forages. College of Food, Agricultural, and Environmental Sciences, Ohio State University Extension. Columbus, Ohio: Ohio State University. [Internet]. 2020 [cited 20 Jul 2025]. Available in: https://goo.su/cpAmPm

[19] Kılıç Ü, Abdiwali MA. Alternatif kaba yem kaynağı olarak şarapçılık endüstrisi üzüm atıklarının *in vitro* gerçek sindirilebilirlikleri ve nispi yem değerlerinin belirlenmesi. Kafkas Üniv. Vet. Fak. Derg. [Internet]. 2016; 22(6):895-901. doi: https://doi.org/qjgj

[20] Suárez-Ramos G, Serrano-Cárdenas V, Pelz-Marín R, Balderas-Aguilar P. Atlas de malezas arvenses del estado de Querétaro. 1ª ed. México: Universidad Autónoma de Querétaro . 2004. 255 p.

[21] Çelik H, Selçuk Z. Karambanın fiğ otu ve yonca otu ile farklı oranda karışımLarının *in vitro* gerçek sindirilebilirliğinin belirlenmesi. Van Vet. J. [Internet]. 2019; 30(3):145-149. doi: https://doi.org/qjj3

[22] Bilal Y, Bakır T, Selçuk B. Evelik otunun (*Rumex acetosella*) kuzu rasyonlarına ilavesinin sindirim derecesine ve fermantasyon parametrelerine etkisinin *in vitro* gaz üretim tekniği ile belirlenmesi. KSÜ Tarım ve Doğa Derg. (Internet). 2023; 26(4):911-918. doi: https://doi.org/qjj4

[23] Ateş E. The mineral, amino acid and fiber contents and forage yield of pea (*Pisum arvense* L.), fiddleneck (*Phacelie tanacetifolia* Benth.) and their mixtures under dry land conditions in the Western Turkey. Romanian Agric. Res. [Internet]. 2012; 29(29):237-244. Available in: https://goo.su/P92N8sF

[24] Martínez-Loperena R, Castelán-Ortega OA, Gonzáles-Ronquillo, M, Estrada-Flores JGE. Nutritive value, *in vitro* fermentation and secondary metabolites of weeds and maize straw used for feeding dairy cattle. Trop. Subtrop. Agroecosyst. [Internet]. 2011; 14(2):525-536. Available in: https://goo.su/d241

[25] Tavendale MH, Lane GA, Schreurs NM, Fraser K, Meagher LP. The effects of condensed tannins from Dorycnium rectum on skatole and indole ruminal biogenesis for grazing sheep. Aust. J. Agric. Res. [Internet]. 2005; 56(12):1331-1337. doi: https://doi.org/d5zxz3

[26] Waghorn GC, Woodward SL. Ruminant contributions to methane and global warming-a New Zealand perspective. In: Bhatti JS, Lal R, Apps MJ, Price MA, editors. Climate Change and Managed Ecosystems. Boca Raton, FL: Taylor & Francis Group; 2006. p. 233-260.

[27] Özkan ÇÖ, Cengiz T, Yanık M, Evlice S. Selçuk B, Ceren B, Kamalak A. Ruminant hayvan beslemede kullanılan bazı kaba ve kesif yemLerin *in vitro* gaz üretiminin, metan üretiminin, sindirim derecesinin ve mikrobiyal protein üretiminin belirlenmesi. Black Sea J. Agric. [Internet]. 2020; 3(1): 56-60. Available in: https://goo.su/Egj9W0J

[28] Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca, NY, USA: Cornell University Press; 1994.

[29] Ece Z, Avcı M. Yonca kuru otu ve süt sığırı rasyonuna zeolit ve meşe palamudu ilavesinin *in vitro* organik madde sindirimi ve metan oluşumu üzerine etkisi. Harran Üniv. Vet. Fak. Derg. [Internet]. 2018; 7(1):67-73. doi: https://doi.org/qjkd

[30] Bach A, Calsamiglia S, Stern MD. Nitrogen metabolism in the rumen. J. Dairy Sci. [Internet]. 2005; 88:E9-E21. doi: https://doi.org/d2h7xj

[31] Clark JH, Klusmeyer TH, Cameron MR. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. [Internet]. 1992; 75(8):2304-2323. doi: https://doi.org/bg2tz6

[32] Bilal Y, Kamalak A. Kuzu rasyonlarına meşe palamudu ilavesinin sindirim derecesine, metabolik enerjisine ve metan üretimine etkisinin *in vitro* gaz üretim tekniği ile belirlenmesi. KSU J. Agric. Nat. [Internet]. 2022; 25(2):583-590. doi: https://doi.org/qjkz

[33] Blümmel M, Lebzien P. Predicting ruminal microbial efficiencies of dairy rations by *in vitro* techniques. Livest. Prod. Sci. [Internet]. 2001; 68(2-3):107-117. doi: https://doi.org/bvq7hk

[34] Vargas JE, López-Ferreras L, Andrés S, Mateos I, Horst EH, López S. Differential diet and pH effects on ruminal microbiota, fermentation pattern and fatty acid hydrogenation in RUSITEC continuous cultures. Fermentation. [Internet]. 2023; 9(4):320. doi: https://doi.org/qjk3

[35] Putri EM, Zain M, Warly ML, Hermon H. Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on *in vitro* digestibility, rumen fermentation, and microbial protein synthesis. Vet. World. [Internet]. 2021; 14(3):640-648. doi: https://doi.org/qjk4

[36] Uddin MD, Haque KZ, Jasimuddin KM, Hasan KMM. Dynamics of microbial protein synthesis in the rumen a review. Ann. Vet. Anim. Sci. [Internet]. 2015 [cited 22 May 2025]; 2(5):116-131. Available in: https://goo.su/gX8abB

[37] Bahrami-Yekdangi M, Ghorbani GR, Khorvash M, Khan MA, Ghaffari MH. Reducing crude protein and rumen degradable protein with a constant concentration of rumen undegradable protein in the diet of dairy cows: Production performance, nutrient digestibility, nitrogen efficiency, and blood metabolites. J. Anim. Sci. [Internet]. 2016; 94(2):718-725. doi: https://doi.org/f8nfm6

[38] Yang C, Bing-Wen S, Qi-Yu D, Hai J, Shu-Qin Z, Yan T. Rumen fermentation and bacterial communities in weaned Chahaer lambs on diets with different protein levels. J. Integr. Agric. [Internet]. 2016; 15(7):1564-1574. doi: https://doi.org/f8wqqw

[39] Paula EM, Monteiro HF, Silva LG, Benedeti BDB, Daniel JLP, Shenkoru T, Broderick GA, Faciola AP. Effects of replacing soybean meal with canola meal differing in rumen-undegradable protein content on ruminal fermentation and gas production kinetics using 2 *in vitro* systems. J. Dairy Sci. [Internet]. 2017; 100(7):5281-5292. doi: https://doi.org/gbkbkn