

Investigating the nutritional value of the red *Alternanthera sessilis* plant for ruminants

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Abstract

The present experiment was conducted to determine the chemical composition, digestibility and fermentation of red *Alternanthera sessilis* and compare its nutritional value with alfalfa either individually or in the form of rations of fattened lamb diets. Four experimental treatments included 1: control (without *Alternanthera sessilis*), 2: ration containing 3% (20% alfalfa substitution), 3: 6% (40% alfalfa substitution) and 4: 9% (60% alfalfa substitution) *Alternanthera sessilis*. Digestibility and fermentation of experimental samples were measured by gas production and two-step digestion test. In terms of chemical composition, there was a great relationship between *Alternanthera sessilis* and alfalfa hay. Adding *Alternanthera sessilis* to feed of lambs significantly increased the amount of truly degraded organic matter, metabolizable energy, and apparent digestibility of organic matter, so that these parameters were highest in treatment containing 9% of *Alternanthera sessilis* plant. On the other hand, by using *Alternanthera sessilis* plant, dry matter digestibility tended to increase. The amount of microbial biomass production and microbial biomass production efficiency were not affected by experimental diets. Rations containing *Alternanthera sessilis* were cheaper than control diets. Therefore, it can be stated that the nutritional value of *Alternanthera sessilis* is comparable to alfalfa hay and can be replaced by alfalfa by up to 60% in the ration of fattening lambs. *In vivo* experiments are also suggested.

Key words: *Alternanthera sessilis*, Alfalfa hay, Digestibility, Ruminants

Introduction

Identifying and determining the nutritional value of new, unconventional, and inexpensive food sources will be useful for feeding food producing livestock. Globally, people, especially those in rural areas and developing countries, rely on wild edible plants, including weeds, to provide staple food, dietary supplements, and medicine (Ulian et al, 2020, Hwong et al, 2022). Weeds are wild plants that can adapt well to various growing conditions. In

general, weeds are unpopular because they are highly invasive and are considered as pests. However, in some cases, weeds are used as food and medicine for humans, in addition to being edible forage for livestock. In this respect, their high adaptability can be useful (Hyde et al, 2021). *Alternanthera sessilis* (anthra meaning flower, silica means no stems, referring to inflorescence) is a medicinal plant with edible leaves. It is a weed

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commonly known as sessile joyweed and also known as dwarf copperleaf or Brazilian spinach (Hyde et al, 2021). The genus *Alternanthera* is an important medicinal plant and member of the Amaranth family containing volatile compounds, essential amino acids, flavonoids, glycosides, and steroids. Medicinal forage herbs and leafy greens such as *Alternanthera sessilis* are consumed as vegetables in Southeast Asia and some other countries (Kumari and Krishnan, 2016). Depending on the color of the aerial part, the *Alternanthera sessilis* can be identified as a green or red cultivar (Othman et al, 2016). Despite being considered a harmful weed in some countries, it has long been used in traditional medicine as well as food sources (Mondal et al, 2014). Information on the chemical composition and nutritional value of the *Alternanthera sessilis* is limited, and its nutritional value for livestock has not been studied. The aim of this study was to determine the nutritional value of this plant for ruminant animals.

Materials and Methods

Experimental design and diets

The experiments were carried out in the research station and laboratories of Agricultural Sciences and Natural Resources University of Khuzestan, Iran. *Alternanthera sessilis*, after cultivation and collection, was dried in oven and was ground with 2 mm sieve mesh for determining the chemical composition and studying the digestion and fermentation.

Measurement of chemical compositions

Alternanthera sessilis, alfalfa hay and other ration ingredients were analysed for crude protein (Kejdahl method, Foss 2033, Denmark), crude fat (Soxhlet method), dry matter, ash, acid detergent fibers (ADF) and

total tannins were measured by standard methods (AOAC, 2012). Determination of neutral detergent fiber (NDF) was done (Van Soest et al., 1991) without the use of α -amylase and sodium sulfite enzyme by removing ash.

Treatments

Alternanthera sessilis was substituted for alfalfa hay at 0 (control), 20, 40, and 60 percent in the diet (equivalent to 3, 6, and 9 percent of the total diet). Diets, composed of 30% forage and 70% concentrate, were fed to fatten lambs based on their live weight (NRC, 2007). *In vitro* digestibility of diets was investigated using gas production and two-step digestion.

Gas production

The rumen fluid was taken from four adult male lambs fed at maintenance level with a forage diet (wheat straw, corn silage, and alfalfa hay). The samples were filtered with a 4-layer cotton cloth and were mixed to be used as the gas production medium. The produced gas was measured using a barometer and glass vials containing buffer and ruminal fluid in a ratio of 2:1. Two hundred mg of dried samples was poured into vials with a 1 mm sieve mesh in 7 replicates. Gas production was recorded at 0, 2, 4, 6, 12, 16, 24, 48, 72, 96 and 120 hours. Gas production coefficients were calculated using the exponential model (Ørskov and McDonald, 1979). Twenty-four-hour time data were used to determine partitioning factors (PF), microbial biomass production, and microbial biomass production efficiency (Blummel et al., 1997). Metabolizable energy (ME), apparent digestibility of organic matter (IVOMAD), and digestible organic matter (DOM) were calculated (Menke et al, 1979).

Table 1: Feed ingredients and chemical composition of the experimental diets fed to lambs

Feed ingredients (%DM)	Percentage of <i>Alternanthera sessilis</i> in ration (Percentage of replacement with alfalfa)			
	Control (0.0)	3 (20)	6 (40)	9 (60)
Alfalfa hay	15.0	12.0	9.0	6.0
<i>Alternanthera sessilis</i>	0	3.0	6.0	9.0
wheat straw	5.0	5.0	5.0	5.0
Corn silage	10.0	10.0	10.0	10.0
Barley grain	35.0	35.0	35.0	35.0
Wheat bran	27.0	27.0	27.0	27.0
Sesame meal	7.0	7.0	7.0	7.0
Vitamins and minerals premix*	0.5	0.5	0.5	0.5
Limestone	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Total	100	100	100	100
Chemical compounds				
Dry matter, %	91.4	91.4	91.4	91.4
Organic matter, % DM	93.6	93.7	93.7	93.8
Crude protein, % DM	14.5	14.3	14.2	14.0
Neutral Detergent fiber, % DM	35.1	34.8	34.6	34.6
Acid Detergent fiber, % DM	17.5	17.4	17.1	17.0
Ash, % DM	6.4	6.3	6.3	6.2
ME (kcal/kg DM)**	2.56	2.57	2.58	2.59

*Each kilogram of supplement contained 600,000 IU of vitamin A, 200,000 IU of vitamin D, 200 mg vitamin E, 2500 mg antioxidant, 195 g calcium, 80 g phosphorus, 21g magnesium, 2200 mg manganese, 3000 mg iron, 300 mg copper, 300 mg zinc, 100 mg cobalt, 12 mg iodine and 1.1 mg selenium.

**Calculated based on the energy density of feed ingredient (Mkcal/kg DM).

Digestibility of diets

In order to determine the *in vitro* digestibility by the two-step digestion method (Tilley and Terry, 1963), 0.5 g of the experimental samples was poured into the test tubes. Rumen fluid was collected from four adult male lambs fed with a forage diet (wheat straw, corn silage, and alfalfa hay) and mixed with artificial saliva with a ratio of 1:4 in 7 replications. After 48 hours of incubation, 6 ml of 20% hydrochloric acid was added to each tube. After that, 0.5 g pepsin was dissolved in 100 ml 0.1 M hydrochloric acid, and 5 ml of this enzyme solution was added to each tube. After 48 hours, the digestibility of dry matter and NDF was calculated based on the amount of the residue. The results were analyzed in a completely randomized design using SAS software and GLM procedure. Significant means were compared using Duncan's multiple-range test at $P < 0.05$. The statistical model was: $Y_{ij} = \mu + T_i + \epsilon_{ij}$ Y_{ij} = observed value, μ =

overall mean, T_i = treatment effect, ϵ_{ij} = error.

Results

The chemical composition of the *Alternanthera sessilis* and alfalfa hay is shown in Table 1. The crude protein content was almost the same in both plants. The NDF and ADF concentrations of alfalfa hay appeared to be higher than those of *Alternanthera sessilis*.

The effect of the experimental diets on gas production parameters are shown in Table 3. By increasing the amount of *Alternanthera sessilis*, gas production potential increased significantly compared to the control ($P < 0.05$) so that the highest and lowest amounts of gas production potential were seen in the diet of 9% *Alternanthera sessilis* plant and the control diet, respectively ($P < 0.05$). The effect of adding *Alternanthera sessilis* on the PF and the truly degradable organic matter was significant ($P < 0.05$), but the effect of

treatments on microbial biomass production efficiency was not significant. The highest truly degradable organic matter was observed in the treatment containing 9% of *Alternanthera sessilis*. The lowest amount of PF was related to 9% treatment and its difference was not significant between the

other treatments. Metabolizable energy (ME), and apparent digestibility of organic matter were significantly affected by the treatments and increased with increasing the amount of *Alternanthera sessilis* so that they were highest in treatment containing 9% of the plant ($P < 0.05$).

Table 2: Chemical composition of *Alternanthera sessilis* and alfalfa hay used in the present experiment

Nutrients	<i>Alternanthera sessilis</i>	Alfalfa hay
Dry matter, %	89.5	87.6
Crude protein, %	13.7	14.2
Neutral detergent fiber, %	42.5	46.65
Acid detergent fiber, %	33.7	37.19
Ash, %	8.1	9.9
Organic matter digestibility (OMD, %)	76.10	72.52
ME (Mcal/kg DM)	2.50	2.34

ME (MJ/kg DM) = $2.2 + 0.1357GP + 0.0057CP + 0.0002859 EE2$ (n=200 / $r^2=0.94$)

DOM% = $0.9042 GP + 0.0492CP + 0.0387CA + 16.49$ (n=85 / $r^2=0.93$)

ME: metabolizable energy, DOM: Digestible organic matter

Table 3: The effect of replacing *Alternanthera sessilis* with alfalfa hay on gas production parameters of experimental rations

Parameter	<i>Alternanthera sessilis</i> in ration, % (Replacement with alfalfa, %)				SEM	P value
	Control (0.0)	3 (20)	6 (40)	9 (60)		
<i>b</i> (ml/h)	112.2 ^c	131.8 ^b	134.4 ^{ab}	145.2 ^a	6.38	0.02
<i>c</i> (ml/h)	0.093 ^b	0.093 ^b	0.097 ^b	0.109 ^a	0.002	0.002
PF, mg/ml	4.77 ^a	4.73 ^a	4.77 ^a	4.36 ^b	0.12	0.004
TDOM, mg	3130.3 ^b	316.7 ^b	326.3 ^a	336.6 ^a	8.28	0.018
MBP, mg	167.18	169.51	175.66	166.65	5.28	0.18
MBPE, %	53.90	53.5	53.8	49.51	2.28	0.42
ME, Mcal/kg DM	3.22 ^b	3.30 ^b	3.36 ^b	3.72 ^a	0.22	0.043
IVOMAD, %	83.35 ^b	85.01 ^b	86.17 ^b	93.93 ^a	3.92	0.042

SEM standard error of means, *b*: Potential of gas production, *c*: gas production rate, PF: Partitioning factor, TDOM: Truly degradable organic matter, MBP: Microbial biomass production, MBPE: Microbial biomass production efficiency, ME: metabolizable energy, IVOMAD: In vitro organic matter apparent digestibility

ME (MJ/kg DM) = $0.04 + 0.1639GP + 0.0079CP + 0.0239EE$

IVOMAD (g/kg DM) = $14.88 + 0.8893GP + 0.0448CP + 0.0651CA$

Means in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 4 shows the volume of gas produced in the diets at different incubation times. At each time, the amount of gas produced increased with increasing of *Alternanthera sessilis*, but the changes were

significant only at 24 and 48 hours ($P < 0.05$). The highest and lowest amount of gas production was seen in the treatment containing 9% of *Alternanthera sessilis* and the control, respectively.

Table 4: The effect of replacing *Alternanthera sessilis* with alfalfa hay on the volume of gas produced of experimental rations at different incubation time

Time (h)	<i>Alternanthera sessilis</i> in ration, % (Replacement with alfalfa, %)				SEM	P value
	Control (0.0)	3 (20)	6 (40)	9 (60)		
24	99.4 ^b	103.1 ^b	105.7 ^{ab}	121.1 ^a	5.44	0.04
48	116.5 ^b	120.1 ^{ab}	122.7 ^{ab}	139.8 ^a	6.94	0.01
72	125.4	129.3	131.9	149.6	7.58	0.13
96	130.1	134.0	136.7	154.3	7.79	0.18

SEM standard error of means

Means in the same row with different superscript letters are significantly different ($P < 0.05$).

In vitro digestibility

The digestibility of dry matter and NDF of the experimental diets are shown in Table 5. There was no difference between the

diets, but the digestibility of treatments containing *Alternanthera sessilis* was numerically better than that of the control.

Table 5: Digestibility (%) of experimental diets containing different levels of *Alternanthera sessilis*

Item	<i>Alternanthera sessilis</i> in ration, % (Replacement with alfalfa, %)				SEM	P value
	Control (0.0)	3 (20)	6 (40)	9 (60)		
Dry matter	70.0	76.9	74.8	77.9	3.33	0.07
Neutral detergent fiber	56.3	56.5	56.8	57.9	1.79	0.68

SEM: standard error of means

Means in the same row with different superscript letters are significantly different ($P < 0.05$).

Discussion

In rumen produced hydrogen can be used during the synthesis of volatile fatty acids and microbial proteins, and its excess is eliminated by methane production by methanogenic microorganisms (Zhu et al, 2021; Snelling et al, 2019; Sirohi et al, 2009). The reason for the improvement of gas production potential in diets containing *Alternanthera sessilis* can be attributed to its chemical composition, i.e. less NDF and ADF content (Table 2) as well as its higher digestibility compared with alfalfa hay (Table 2). This relationship has been proven in literature so that increasing the concentration of cell walls (NDF and ADF) reduces non-fiber carbohydrates (NFC) and soluble sugars and ultimately reduces digestibility, fermentation, and gas production (Coblentz et al, 2013). The gas produced is greatly influenced by the chemical composition and physical properties of food (He et al, 2020) which may be partly due to changes in the activity of rumen microorganisms (He et al, 2020). The researchers examined the relationship between the chemical composition of different parts of 13 maize varieties with fermentation, digestion, and gas production; the results showed that this correlation was particularly high in the stem section (He et al, 2020).

In Table 2, it was observed that the *Alternanthera sessilis* plant has more ME than alfalfa fodder, perhaps due to the production of more gas (Tables 3 and 4) and organic matter digestibility (Table 2) than

alfalfa hay. In this context, the researchers suggested that there was a high correlation between metabolizable energy and gas produced in 24 hours and the chemical composition of food (Menke et al, 1979; Bakhshizadeh, 2014). High levels of gas produced indicate high metabolizable energy, as well as fermentable nitrogen and other nutrients necessary for microorganisms to function (Newbold et al, 2015). In an experiment, the reduction of gas production in different levels of Gopar (*Heracleum persicum*, with flavonoid compounds such as *Alternanthera sessilis*) has been reported (Nooriyan Soroor and Rouzbehan, 2013). Many studies have reported that the use of medicinal plants or their active ingredients reduces the digestibility of feed during incubation (Garcia-Gonzalez et al., 2006) followed by a decrease in gas production. In a study, the use of different levels of *Echium amoenum* (containing flavonoids such as *Alternanthera sessilis*) increased the amount of gas produced in the diet (Nooriyan Soroor and Rouzbehan, 2014) which was in agreement with the results of this study. The addition of different amounts (0, 10, 20, 30, and 40 mg) of extracted flavonoid from *Alternanthera sessilis* to diets containing different ratios of rice straw and concentrate (50, 60, and 70 percent concentrate) showed that in diets containing 50 and 60 percent concentrate, gas production up to 30 mg flavonoid levels was higher than control, in other words,

adding flavonoid increased gas production. On the other hand, regardless of the base diet (rice straw to concentrate ratio), adding flavonoids to control increased gas production by 10 mg and then was unchanged at a 30 mg level and decreased at 40 mg compared to the control (Sommai et al. 2021); these results were consistent with the results of the present experiment and confirmed the effect of flavonoids in *Alternanthera sessilis*.

The partitioning factor is the ratio of the truly degradable organic matter to the volume of the gas produced, reflecting a variation in the production of short-chain fatty acids per unit of the degraded substrate (Blummel et al, 1997; Souhil et al, 2022). It has been reported that the PF calculated under *in vitro* conditions creates significant data for predicting dry matter consumption, production of microbial biomass in the rumen, and methane emissions in ruminants (Noviandi et al, 2021). Therefore, part of the difference in the PF and the microbial biomass production efficiency among the treatments in this experiment is due to the different gas production (Tables 3 and 4). According to the results, the amount of truly degradable organic matter in treatments containing *Alternanthera sessilis* increased compared to the control treatment. By comparing the results and investigating the relationship between gas and PF, it is clear that with increasing the gas production (Table 4), the PF has increased. In fact, lower levels of PF indicate low microbial protein production efficiency. This means that a larger share of the digested feed has been spent on gas production than microbial protein synthesis (Sallam et al, 2009). Also, researchers have stated that with increasing gas production (methane), partitioning factor decreases and the increase in PF in their study indicates an improvement in fermentation efficiency (Souhil et al, 2022). Also, the reduction of PF in treatment of 9% compared to control is probably due to synchronization of energy and protein release in rumen due to some chemical

compounds in the plant (Jimenez-Peralta et al, 2011). It was reported that the using of *Echium amoenum* plant increased PF and biomass production efficiency (Nourian sarvar and Rouzbahan, 2014). In another study (Nooriyan Soroor and Rouzbahan, 2013), the researchers observed the positive effect of Golpar (*Heracleum persicum*) on increasing the amount of these parameters. The researchers reported that reductions in dry matter degradability, gas, and methane production could be attributed to the antimicrobial activity of flavonoids (Cushnie and Lamb, 2011); but in the present experiment, although the *Alternanthera sessilis* plant contains flavonoids (Sommai et al, 2021), did not have a negative effect on fermentation and digestion, which can be attributed to the flavonoid level of plants or diets according to previous studies (Sommai et al, 2021). In their experiment, the use of 10 and 20 mg of flavonoid extracted from the *Alternanthera sessilis* plant increased gas production and remained unchanged until 30 mg and then decreased at 40 mg level, compared to the control (Sommai et al, 2021). Patra and Saxena (2010) observed a decrease in carboxy methyl cellulase and xylanase activity of rumen bacteria in the presence of high concentrations of clove and fennel extracts (containing quercetin, such as the *Alternanthera sessilis* plant) which could also be another reason for the reduction of PF in levels of 9%.

The results showed that *Alternanthera sessilis* plant is very similar to alfalfa hay in terms of chemical composition. The use of *Alternanthera sessilis* as part of fattening lambs' diets not only had no significant negative effect on *in vitro* digestion and fermentation parameters but in several cases resulted in improved digestibility and fermentation of diets. In order to complete the information about the effects of feeding this plant to animals, it is better to conduct *in vivo* experiments on animals such as fattening lambs or lactating animals, etc.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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بررسی ارزش تغذیه‌ای گیاه *آلترنانترا سیسیلیس* (*Alternanthera sessilis*) قرمز برای نشخوارکنندگان

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چکیده

آزمایش حاضر با هدف تعیین ترکیب شیمیایی و قابلیت هضم و تخمیر گیاه *آلترنانترا سیسیلیس* قرمز و مقایسه ارزش تغذیه‌ای آن با یونجه به صورت انفرادی یا در قالب جیره‌های بره‌های پروراری انجام شد. چهار تیمار آزمایشی شامل ۱- شاهد (فاقد *آلترنانترا سیسیلیس*)، ۲- جیره حاوی ۳ درصد (۲۰ درصد جایگزینی با یونجه)، ۳- ۶ درصد (۴۰ درصد جایگزینی با یونجه) و ۴- ۹ درصد (۶۰ درصد جایگزینی با یونجه) *آلترنانترا سیسیلیس* بود. قابلیت هضم و تخمیر نمونه‌های آزمایشی با روش تولید گاز و آزمایش هضم دومرحله‌ای سنجش شد. قرابت زیادی از نظر ترکیب شیمیایی بین *آلترنانترا سیسیلیس* با یونجه وجود داشت. افزودن گیاه *آلترنانترا سیسیلیس* به جیره بره‌های پروراری باعث افزایش معنی‌دار مقدار ماده آلی واقعا تجزیه شده، انرژی قابل متابولیسم، انرژی خالص شیردهی و قابلیت هضم ظاهری ماده آلی شد؛ به طوری که این شاخص‌ها در تیمار حاوی ۹ درصد گیاه *آلترنانترا سیسیلیس* بیش‌ترین مقدار بودند. از طرفی، با استفاده از گیاه *آلترنانترا سیسیلیس* در جیره قابلیت هضم ماده خشک تمایل به افزایش نشان داد. مقدار تولید توده زنده میکروبی، بازده تولید توده زنده میکروبی تحت تأثیر جیره‌های آزمایشی قرار نگرفتند. قیمت جیره‌های حاوی گیاه *آلترنانترا سیسیلیس* ارزان‌تر از جیره شاهد بود. بنابراین می‌توان بیان کرد که ارزش تغذیه‌ای گیاه *آلترنانترا سیسیلیس* با علوفه یونجه قابل مقایسه است و می‌توان آن را تا ۶۰ درصد با یونجه در جیره بره‌های پروراری جایگزین کرد. انجام آزمایش‌های دامی نیز توصیه می‌شود.

کلمات کلیدی: *آلترنانترا سیسیلیس*، علوفه یونجه، قابلیت هضم، نشخوارکنندگان

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