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The effect of replacing alfalfa hay with sesbania straw (*Sesbania sp*. L) on the digestive and fermentation activity of anaerobic bacteria and fungi isolated from the rumen of sheep

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Abstract The purpose of this research was to compare the nutritional value of sesbania straw with alfalfa hay and determine its effects on the digestive and fermentation activity of the ruminal anaerobic bacteria and fungi. The experimental diets included a control diet (without sesbania straw) and diets in which alfalfa hay was replaced with sesbania straw at the rate of 25, 50, 75, and 100% in a fattening lamb diet. The *in vitro* digestibility of dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) was the highest (P<0.05) in the control diet (P<0.05), but not different from the diets containing 25, 50 and 75% of the sesbania straw in lieu of alfalfa hay (P>0.05). The digestibility of dry matter, NDF, and ADF of alfalfa hay was higher than that of sesbania straw (P<0.05). Compared to the control diet, the replacement of sesbania plant with alfalfa hay up to 75% level did not have a significant effect on the digestibility of DM, NDF, and ADF by isolated rumen bacteria and fungi, but at 100% replacement, the digestibility decreased (P<0.05). Also, in the culture medium of isolated rumen bacteria and fungi, alfalfa hay had more digestibility of the nutrients than sesbania straw (P<0.05). The pH and ammonia nitrogen concentration of the culture medium containing the isolated rumen bacteria and fungi were significantly higher in the control diet than in the diets containing sesbania straw (P<0.05). In general, the findings showed that it is possible to replace at least 75% of alfalfa hay with sesbania straw in the diets of fattening lambs.

Keywords: ammonia nitrogen, digestibility, microbial culture, pH, sesbania

Introduction

Considering the limitations in forage and water resources and the high price of feed ingredients, the use of affordable forage resources is very important because a significant part of animal production costs is feed. Therefore, improving feed efficiency can be the most important factor in reducing the costs of raising livestock, which is more important when feed costs increase or the value of livestock products decreases (Ellison et al., 2017). On the other hand, the global growth of the human population has increased the demand for meat and milk, which has caused significant public concerns (Moorby and Fraser, 2021).

There are roughage sources in Iran, which may contribute to nutritional needs of livestock, but their protein, mineral, and digestible energy contents are of little value for livestock due to their lignocellulosic structure. Therefore, the use of high-protein plants not only serves as an appropriate feed for livestock but also enhances the quality and nutritional value of low-quality feeds (Ramírez-Briones et al., 2017).

Rumen microorganisms play an important role in the digestion of feed and this affects the efficiency of feed utilization (Ellison et al., 2017).

Sesbania rostrata, a multi-purpose plant native to regions such as India and Malaysia, is cultivated as forage and green manure in Southeast Asia before rice. According to reports, sesbania plant has the ability to grow rapidly. It could be beneficial to cultivate it as green manure before planting rice, as it is believed to increase the yield of the rice crop by 20-40%. Additionally, using sesbania as a green manure improves the quality of poor soils by providing organic matter (OM) to soils (Mutisya et al., 2014).

Many researchers have reported the presence of carbohydrates, glycosides, proteins, amino acids, saponins, tannins, alkaloids, phenolic compounds and flavonoids in sesbania, and the pods and leaves of this plant contain cholesterol and beta-sitosterol (Mani et al., 2011). The sesbania seeds contain oleanolic acid, galactopyranoside, and galactomannan. Cyanidin, alpha-ketoglutaric, oxaloacetate, and pyruvic acids are present in sesbania flowers. Calcium and phosphorus have also been reported in sesbania (Goswami et al., 2016). Sesbania is a highly adaptable plant that can thrive in adverse soil conditions, including poor texture, drainage, and nutrition. Additionally, it has the ability to regenerate after each harvest, making it a suitable fodder plant for regions characterized by poor and relatively salty soils (Dareini et al., 2017).

Some studies have shown that inclusion of the sesbania plant in livestock diets can lead to reduced feed costs, improved feed consumption, increased digestibility, and better feed conversion ratio (Zaki et al., 2015). Sesbania plant can also affect ruminal fermentation and milk production in livestock (Goswami et al., 2016). It is highly nutritious and has similar nutritional value to alfalfa hay, vetch, and other oilseeds (Farghaly et al., 2022). This plant is an excellent alternative to alfalfa for livestock, particularly small ruminants, due to its high digestibility, low fiber content, and high protein content (Gebreyowhans and Zegeye, 2019). It is not advisable to feed monogastric animals like chickens, rabbits, and pigs with sesbania due to the presence of certain anti-nutritional compounds; additionally, ruminants should not be fed with certain species of sesbania straws in quantities exceeding 10-20% of their diet (Salehi, 2016).

Based on several studies, it appears that the sesbania plant holds great potential as animal feed due to high nutritional value. However, when it comes to feeding ruminants, it is crucial to consider the impact of the feed on the population of ruminal microorganisms. These microorganisms are vital for the proper digestion and fermentation of feedstuff. This is especially important in the case of sesbania plant since it contains numerous secondary metabolites such as glycosides, saponins, tannins, alkaloids, phenolic compounds, flavonoids, cyanidins, oxaloacetic acid, galactopyranoside, and galactomannan. These compounds may have varying effects on the population of rumen microorganisms, potentially impacting their digestive and fermentation capacity (Mani et al., 2011; Goswami et al., 2016).

The impact of sesbania plant on the digestive and fermentation activity of anaerobic bacteria, and fungi isolated from the rumen of fattening lambs, was investigated in the present experiment. The lack of information about the effect of sesbania plant on the population of ruminal microorganisms prompted this study.

Materials and methods

All experimental procedures were carried out in Agricultural Sciences and Natural Resources University of Khuzestan (ASNRUKH) according to the Care and Use of Agricultural Animals in Research and Teaching guidelines (FASS, 2010), and the study was approved by the ASNRUKH Animal Care Committee (Approval no ASD 010/1398). The tested sesbania straw was taken from the Agricultural Research and Education Center and Natural Resources of Safiabad, Dezful, Iran. In the present experiment, the remains of cultivated sesbania were used to prepare the seeds; therefore, after separating the seeds, the nutritional value of the remains was investigated.

Treatments and experimental diets

The study involved five diets for fattening lambs (NRC, 2007), each containing varying amounts of sesbania straw as a replacement for alfalfa hay. The experimental diets included: 1) a control diet without sesbania, 2) a diet with 25% replacement (7.5% of the total diet), 3) a diet with 50% replacement (15% of the total ration), 4) a diet with 75% replacement (22.5% of the total diet), and 5) a diet with 100% replacement of sesbania with alfalfa hay (30% of the total diet) as shown in Table 1. Moreover, the study included a comparison of the sesbania straw and alfalfa hay.

In vitro digestibility

The digestibility of the experimental samples was measured using a two-stage digestion experiment (Tilley and Terry, 1963). Ruminal fluid was prepared from three adult male Arabi sheep (40±3.5 kg weight; 30±4 months old), and mixed with artificial saliva at a ratio of 1 to 4. The amount of 0.5 g dry sample was weighed in 100 mL tubes (7 replicates for each sample) and incubated with the above solution for 48 hours under anaerobic conditions at 39°C. Then, after adding 20% hydrochloric acid and pepsin (0.5 g of pepsin enzyme 3300 in 100 mL of 0.1 M hydrochloric acid), it was incubated for another 48 hours at 39°C. The samples were then filtered using ashless filter paper (Whatman No. 42), and the remaining matter was dried and weighed. The digestibility of DM, NDF, and ADF was calculated from the difference between the amount of the initial and residual weight of samples (Ungerfeld, 2020).

Table 1. Feed ingredients and chemical composition of the experimental diets (% of DM)

	Treatments (% sesbania straw replacing alfalfa hay)				
Feed	Control (0%)	25%	50%	75%	100%
Ingredients					
Alfalfa hay	30.0	22.5	15.0	7.5	0.0
Sesbania straw	0.0	7.5	15.0	22.5	30.0
Wheat straw	20.0	20.0	20.0	20.0	20.0
Corn grain	3.5	3.5	3.5	3.5	3.5
Barley grain	20.0	20.0	20.0	20.0	20.0
Soybeans meal	5.0	5.0	5.0	5.0	5.0
Wheat bran	20.0	20.0	20.0	20.0	20.0
Vitamin and mineral Premix[1]	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5
Chemical composition (%)					
Dry matter	86.04	86.34	86.87	86.53	87.83
Crude protein	14.68	14.83	14.98	15.13	15.28
Neutral detergent fiber (NDF)	41.04	40.24	39.44	38.64	37.84
Acid detergent fiber (ADF)	25.9	24.86	23.82	22.82	21.74
Ash	7.30	7.20	7.11	7.01	6.92
Non-fiber carbohydrates (NFC)[2]	32.98	33.73	34.47	35.22	35.96
Total tannin	0.0	0.37	0.75	1.12	1.50
Metabolizable energy (ME, Mcal/kg)[3]	2.35	2.40	2.45	2.50	2.55

[1]Premix contained (per kg): Vitamin A, 500,000 IU/mg; vitamin D3, 100000 IU/mg; vitamin E, 100 mg/kg; Ca, 180 g/kg; P, 60000, mg/kg; Na, 60000 mg/kg; Mg, 19000 mg/kg; Zn, 3000 mg/kg; Fe, 3000 mg/kg; Mn, 19000 mg/kg; Cu, 300 mg/kg; Co, 100 mg/kg; Se, 1 mg/kg; I, 100 mg/kg; antioxidant, 400 mg/kg; carrier, up to 1000 g.
[2] NFC= 100% - (CP% + NDF% + EE% + Ash%)
[3] ME (ME, MJ/kg) = 0.04 + 0.1639GP + 0.0079CP + 0.0239EE, where, GP is gas production, which measured by gas production technique (Menke and Steingass, 1988).

Special culture medium for rumen fungi

The culture medium for rumen fungi was prepared by mixing 150 mL of salty solution 1 (3 grams of dipotassium hydrogen phosphate dissolved in one liter of distilled water), 150 mL of salty solution 2 (3 grams of potassium hydrogen phosphate, 6 grams of ammonium sulfate, 6 grams of sodium chloride, and 0.6 grams of calcium chloride dissolved in one liter of distilled water), 150 mL of centrifuged rumen liquid (spun at 15000 rpm for 30 minutes), 2.5 grams of yeast extract, 10 grams of peptone trypticase, 0.5 grams of glucose, one gram of cellobiose, 6 grams of sodium bicarbonate, one gram of cysteine-HCL, and one milliliter of 0.1% resazurin per liter of the culture medium (Orpin, 1977; Mohammadabadi et al., 2012).

The special culture medium for fungi was prepared by transferring it to serum bottles in anaerobic conditions and then autoclaving for 15 minutes at 120°C. The fungi isolates were cultured as inoculants at 1:9 ratio in the serum bottles containing the special culture medium, along with one gram of experimental samples and one milliliter of antibiotics (consisted of 0.01 grams of penicillin and streptomycin, and 2 milliliters of chloramphenicol brought to a volume of 100 milliliters). Three stages of subculture were performed to obtain a pure culture medium. After ensuring that the fungal culture medium was free of any contaminants, the samples were placed in an incubator at a temperature of 39°C for six days (Orpin, 1977; Mohammadabadi et al., 2012). The pH value and ammonia nitrogen (NH_3-N) concentration were measured at the end of each incubation time, which included the first, third, and sixth days of incubation.

In addition, the content of the culture medium for fungi was dried in an oven at 90°C for 24 hours. The percentage disappearance of DM, NDF, and ADF of the samples was calculated from the difference of the initial and, and residual weights. The mean percent digestibility was reported, regardless of the incubation time.

Culture medium for rumen bacteria

To prepare the culture medium for rumen bacteria, 150 mL of salty solution 1 (6 grams of dipotassium hydrogen phosphate dissolved in one liter of distilled water) and 150 mL of salty solution 2 (3 grams of potassium hydrogen phosphate, 6 grams of ammonium sulfate, 0.5 grams of magnesium sulfate, 0.6 grams of sodium chloride, and 0.2 grams of calcium chloride dissolved in one liter of distilled water) were combined. The solution was then mixed with 0.5 grams of yeast extract, 70 mL of 8% sodium carbonate, 10 mL of volatile fatty acids, and one milliliter of 0.1% resazurin (Caldwell and Bryant, 1966). The materials were mixed together and distilled water was added to reach a total volume of one liter. The resulting mixture was boiled before adding the 0.1% reduction solution (which contained cysteine-HCl and sodium sulfide, $9H_2O$). Finally, the reduction solution was added to the culture medium. The culture medium prepared under anaerobic conditions was transferred into serum bottles containing one gram of test samples. The bottles were autoclaved at 120°C for 15 minutes. After that, 5 mL of pure rumen bacteria inoculant, obtained by centrifuging fresh rumen fluid, and 3 mL of

1.5% sugar solution (glucose) were added to the bottles. To obtain a pure culture medium, three stages of subculture were performed (Hashemi et al., 2018; Caldwell and Bryant, 1966).

After purifying, the cultures were incubated in an incubator at a temperature of 39°C. The pH was measured and the culture liquid was sampled to measure NH_3-N at the end of each incubation period, which was 24, 48, and 72 hours. Subsequently, the contents of the culture bottles were dried and the disappearance of DM, NDF, and ADF was measured. The average percentage of nutrient digestibility was reported regardless of the incubation time (Hashemi et al., 2018; Caldwell and Bryant, 1966).

Ammonia nitrogen concentration and pH

To measure the concentration of NH_3-N after incubation, a portion of the liquid culture medium was mixed with an equal volume of 0.2 normal hydrochloric acid. Concentration of NH_3-N was measured using a spectrophotometer (Bio-Rad, Libra S22, England) (Broderick and Kang, 1980). To examine the effect of experimental diets on pH changes in the culture medium of bacteria and fungi isolated from the rumen, pH was measured using a pH meter (model WTW 3110, made in Germany).

Chemical composition

The percentage of DM was measured in an oven (Memmert, Germany) at 60°C for 48 hours (Method: 92.05). The ADF and NDF were measured according to AOAC (2012) (method 973.18) and Van Soest et al. (1991), respectively.

Statistical analysis

Data were analyzed using the statistical software SAS version 9.4 in a completely randomized design using the Proc GLM. The comparison of means was done at the error 5% level using Duncan's multiple range test. The statistical model of the design was as follows:

$Y_{ij}=\mu+ T_i+ \varepsilon_{ij}$

In this model, Y_{ij} was the measured value of each observation, μ was the population mean, T_i was the treatment effect, and ε_{ij} was the statistical population error.

Results

Chemical composition

Chemical composition of *Sesbania Sp.* and alfalfa hay are presented in Table 2. Concentration of DM, CP, NDF, ADF, ash and OM, tannin, and metabolizable energy (ME) in sesbania straw were 84.32, 18, 25.2, 15, 8.69, 91.31, 3, and 3.28 percent, respectively. The ash, NDF, ADF, and lignin contents of the sesbania straw were 1.28, 17.67, and 18.88, and 3.11 percent, lower than alfalfa hay, respectively. In contrast, sesbania straw comapred to alfalfa hay, contained higher levels of CP, OM, and ME (2 and 1.28%, and 0.12 Mcal, respectively).

Table 2. Chemical composition of sesbania straw and alfalfa hay

Chemical composition	Sesbania straw	Alfalfa hay
Dry matter (%)	84.32	94.36
Crude protein (%)	18.00	16.00
Organic matter (%)	91.31	90.03
Neutral detergent fiber (%)	25.20	42.87
Acid detergent fiber (%)	15.00	33.88
Lignin (%)	6.39	9.50
Ash (%)	8.69	9.97
Metabolizable energy **(**Mcal/kg)[1]	3.28	3.16
Total tannin (%)	3.00	-
Total phenols (%)	4.50	-
Condensed tannins (%)	2.12	-
Phytic acid (%)	2.17	-
Saponins (%)	1.26	-

[1] ME (MJ/kg) = 0.04 + 0.1639GP + 0.0079CP + 0.0239EE, where, GP is gas production, where, GP is gas production, which measured by gas production technique (Menke and Steingass, 1988).

In vitro digestibility

Table 3 presents the results on the diet nutrients digestibility. The control diet showed the highest DM, NDF, and ADF digestibility, while the diet with the highest (100%) replacement of the sesbania straw with alfalfa hay showed the lowest digestibility (P<0.05). However, there was no significant difference in digestibility of these nutrients between the control and diets containing 25, 50, and 75% sesbania. The digestibility of DM, NDF, and ADF in alfalfa hay was higher than that sesbania straw (Table 4).

Table 3. The effect of sesbania straw replacing alfalfa hay on the *in vitro* digestibility (%; two-stage digestion) of the diets

Parameters	Treatments (% sesbania straw replacing alfalfa hay)					SEM	P-value
	Control (0%)	25%	50%	75%	100%		
Dry matter	64.55[a]	64.20[a]	63.92[a]	63.77[ab]	62.32[b]	0.72	0.0010
Neutral detergent fiber	62.08[a]	61.58[a]	61.47[a]	60.51[ab]	60.09[b]	0.501	0.0001
Acid detergent fiber	58.38[a]	58.16[ab]	57.31[ab]	57.73 [ab]	57.12[b]	0.58	0.0001

SEM: standard error of the mean

a,b: Within row, mean values with common superscript (s) are not different (P>0.05).

Digestibility of nutrients in the rumen bacteria culture medium

The digestibility of DM, NDF, and ADF in diets that were incubated with rumen anaerobic bacteria was influenced by the experimental diets ($P<0.05$). Up to the 75% level of alfalfa replacement with sesbania, the digestibility of these nutrients were not significantly different from the control diet (Table 5). The lowest nutrient digestibility was observed in the 100% replacement diets, while the highest percentage was recorded in the control diet ($P<0.05$). However, in diets with 25%, 50%, and 75% substitution, the digestibility of nutrients was not significantly different from the control diet.

Table 4. The *in vitro* digestibility (%) of alfalfa hay and sesbania straw

	Treatments			
Parameters	Alfalfa hay	Sesbania straw	SEM	P-value
Dry matter	64.14^{a}	58.65^{b}	0.72	0.001
Neutral detergent fiber	60.10^{a}	55.39^{b}	1.192	0.029
Acid detergent fiber	56.91^{a}	53.73^{b}	0.62	0.040

SEM: standard error of the mean
a,b: Within row, mean values with common superscript (s) are not different (P>0.05).

Table 5. The effect of substitution of alfalfa hay with sesbania straw on the nutrient digestibility (%) and fermentation parameters in the specific culture medium of rumen bacteria

		Treatments (% sesbania straw replacing alfalfa hay)						
Parameters	Time (hours)	Control	25%	50%	75%	100%	SEM	P-value
Digestibility (%)								
Dry matter	-	64.23^{a}	63.70^{ab}	63.33^{ab}	62.14^{ab}	62.46^{b}	0.79	0.0046
NDF	-	58.32^{a}	57.52^{ab}	57.58^{ab}	57.33^{ab}	56.84^{b}	0.64	0.0370
ADF	-	49.43^{a}	48.80^{ab}	48.60^{ab}	47.49^{ab}	47.38^{b}	0.86	0.0001
Fermentation parameters								
pH	24	6.14^{a}	5.61^{b}	5.51^{b}	5.47^{b}	5.44^{b}	0.11	0.0007
NH_3-N (mg/dL)	24	6.80^{a}	5.70^{a}	5.69^{b}	4.59^{c}	4.34^{c}	0.186	0.0001
pH	48	5.92	5.60	5.53	5.51	5.50	0.177	0.4309
NH_3-N (mg/dL)	48	6.47^{a}	6.48^{a}	5.38^{b}	5.24^{b}	4.71^{c}	0.101	0.0001
pH	72	6.06^{a}	5.88^{ab}	5.84^{ab}	5.64^{b}	5.26^{c}	0.130	0.0027
NH_3-N (mg/dL)	72	6.23^{a}	6.12^{a}	5.23^{b}	4.79^{c}	4.20^{d}	0.065	0.0001

SEM: standard error of the mean
a,b: Within row, mean values with common superscript (s) are not different (P>0.05).

Data on the digestibility parameters in the culture medium of bacteria isolated from the rumen (Table 6) indicated that alfalfa hay recorded higher digestibility of DM, NDF, and ADF compared to the sesbania straw ($P<0.05$).

Table 6. Comparison of the nutrient digestibility (%) and fermentation parameters of alfalfa hay with sesbania straw in the specific culture medium of rumen bacteria

		Treatments			
Parameters	Time (hours)	Alfalfa hay	Sesbania straw	SEM	P-value
Digestibility (%)	-				
Dry matter	-	60.18^{a}	53.50^{b}	1.89	0.0046
NDF	-	56.10^{a}	51.62^{b}	0.30	0.0001
ADF	-	53.75^{a}	48.09^{b}	1.94	0.0019
Fermentation parameters					
pH	24	6.55^{a}	5.47^{b}	0.146	0.0001
NH_3-N (mg/dL)	24	8.43^{a}	4.47^{b}	0.146	0.0001
pH	48	6.05	5.92	0.0333	0.4294
NH_3-N (mg/dL)	48	8.43^{a}	4.34^{b}	0.121	0.0001
pH	72	5.97	6.06	0.2457	0.8043
NH_3-N (mg/dL)	72	8.25^{a}	4.46^{b}	0.077	0.0001

SEM: standard error of the mean
a,b: Within row, mean values with common superscript (s) are not different (P>0.05).

Digestibility of nutrients in rumen fungi culture medium

The DM digestibility in the presence of the rumen anaerobic fungi (Table 7) was lowest at 100% replacement of alfalfa hay with sesbania straw and highest in the control ($P<0.05$); there was no difference between control and other treatments.

The replacement of alfalfa with sesbania did not affect the digestibility of NDF and ADF of the experimental diets by fungi isolated from the rumen. However, the nutrient digestibility of alfalfa in diets incubated with rumen anaerobic fungi was significantly higher than that of sesbania (Table 8).

Table 7. The effect of replacing alfalfa hay with sesbania straw on the nutrient digestibility (%) and fermentation parameters in the specific culture medium of rumen fungi

		Treatments (% sesbania straw replacing alfalfa hay)						
Parameters	Time (days)	Control	25%	50%	75%	100%	SEM	P-value
Digestibility (%)	-							
Dry matter	-	61.06^{a}	60.65^{ab}	60.30^{ab}	59.88^{ab}	59.63^{b}	0.59	0.012
NDF	-	52.46	52.44	52.43	52.05	51.76	0.38	0.123
ADF	-	45.22	44.95	44.77	44.55	44.49	0.42	0.201
Fermentation parameters								
pH	First	5.46^{a}	5.27^{ab}	5.26^{ab}	5.17^{b}	5.13^{b}	0.0664	0.0181
NH_3-N (mg/dL)	First	6.62^{a}	5.96^{b}	5.54^{c}	4.72^{d}	4.41^{d}	0.108	0.0001
pH	Third	5.56^{a}	5.48^{ab}	5.31^{cb}	5.28^{c}	5.16^{c}	0.0596	0.0005
NH_3-N (mg/dL)	Third	6.36^{a}	6.17^{a}	5.39^{b}	4.44^{c}	4.36^{c}	0.9991	0.0001
pH	Sixth	5.53	5.43	5.42	5.37	5.32	0.0663	0.3041
NH_3-N (mg/dL)	Sixth	6.63^{a}	6.61^{a}	5.62^{b}	5.19^{c}	5.19^{d}	0.124	0.0001

SEM: standard error of the mean

a,b: Within row, mean values with common superscript (s) are not different (P>0.05).

Table 8. Nutrient digestibility (%) and fermentation parameters of alfalfa hay and sesbania straw in the specific culture medium of rumen fungi

		Treatments			
Parameters	Time (days)	Alfalfa hay	Sesbania straw	SEM	P-value
Digestibility (%)	-				
Dry matter	-	60.05^{a}	55.66^{b}	1.89	0.0001
NDF	-	54.25^{a}	51.75^{b}	0.23	0.0004
ADF	-	47.35^{a}	44.76^{b}	0.28	0.0001
Fermentation parameters					
pH	First	5.54^{a}	5.15^{b}	0.060	0.0035
NH_3-N (mg/dL)	First	8.58^{a}	5.37^{b}	0.112	0.0001
pH	Third	5.93	5.42	0.157	0.6260
NH_3-N (mg/dL)	Third	8.53^{a}	5.26^{b}	0.071	0.0001
pH	Sixth	5.65^{a}	5.28^{b}	0.082	0.0178
NH_3-N (mg/dL)	Sixth	9.06^{a}	6.83^{b}	0.254	0.0003

SEM: standard error of the mean

a,b: Within row, mean values with common superscript (s) are not different (P>0.05).

Fermentation parameters of rumen anaerobic bacteria culture medium

The pH and NH_3-N concentration of the culture medium containing rumen bacteria (Table 5) were significantly affected by the experimental diets (P<0.05). During the first, second, and third days (24 to 72 hours), replacing alfalfa hay with the sesbania straw significantly decreased the pH and NH_3-N concentration of the culture medium compared to the control. Additionally, an increase in the amount of sesbania resulted in further decrease (numerical or significant) in pH and NH_3-N concentration. The control had the highest pH and NH_3-N concentration, whereas the 100% substitution treatment had the lowest. However, the pH on the second day was not affected by the experimental diets and showed a numerical decrease (P>0.05).

Comparison of sesbania and alfalfa incubated with rumen anaerobic bacteria showed that pH and NH_3-N concentration in sesbania were lower than in alfalfa (Table 6). This difference was significant for NH_3-N concentration at all incubation times. The pH difference of the specific culture medium of rumen bacteria on the second and third days between sesbania and alfalfa was non-significant.

Fermentation parameters of specific culture medium of rumen anaerobic fungi

The pH and NH_3-N concentration of the culture medium containing rumen fungi (Table 7) were significantly affected by the experimental diets (P<0.05). The results related to the pH and NH_3-N concentration of the culture medium of rumen anaerobic fungi on the first, third, and sixth days indicate that the presence of sesbania in the diet caused a significant or numerical reduction of these parameters compared to the control. The control diet had the highest pH value and NH_3-N concentration, while the 100% substitution diet had the lowest pH value and NH_3-N concentration. On the third day, the experimental diets did not have a significant effect on pH (P<0.05). However, NH_3-N concentration was significantly affected by the experimental diets at all incubation times and was the highest in the control diets (P<0.05).

The pH and NH_3-N concentration of sesbania, when incubated with anaerobic fungi, were lower than alfalfa on the first, third, and sixth days (Table 8). However, the pH difference on the third day was not significant.

Discussion

Researchers reported that the amount of CP of sesbania species including *Sesbania aculeata* and *Sesbania rostrata* is 25.4% and 32.7%, respectively, and its fiber percentage is 16% and 15.5%, respectively (Shahjalal and Topps, 2000). The protein and fiber content of another species of plant called agasti (*Sesbania grandiflora*) has been reported as 35.00% and 11.93%, respectively. Additionally, some studies have indicated that the sesbania straw contains 11.22% CP, 13.83% NDF, and 8.59% ash, (Dareini et al., 2018). The chemical composition of the sesbania straw in the present study was in agreement with the previous studies to an acceptable extent. The reason for the difference in the chemical composition of this plant in different experiments is related to several factors such as the type of plant species, geographical location, and environmental and ecological factors, such as temperature, rainfall, soil type, and the like (Karimi et al., 2020). In addition, in the present experiment, the remains of sesbania after the separation of seeds were used; but in other experiments mostly sesbania hay has been used; therefore, the lower amount of CP or more fibers in the present experiment could be the reason.

We found that substituting 75% of alfalfa hay with sesbania straw in the diet did not significantly affect the digestibility of nutrients when compared to the control diet. This could be attributed to sesbania having lower ADF, NDF, and lignin content than alfalfa hay (Table 2). Perhaps the decrease in digestibility of nutrients in sesbania compared to alfalfa or at full replacement (100 percent) is related to the secondary metabolites present in sesbania (Table 2). The presence of saponins, tannins, alkaloids, phenolic compounds, and flavonoids has been reported in the sesbania plant (Mani et al., 2011). Rumen microorganisms are capable of breaking down saponin, a glycosidic substance, into aglycone and sugar components. However, the aglycone portion is highly complex and cannot be degraded by rumen bacteria. To date, there have been no reports of rumen bacteria degrading aglycone (Kregiel et al., 2017). Several studies have shown that saponins can decrease the digestibility of certain dietary nutrients including NDF (Liu et al., 2019). However, in some studies, saponin did not affect the digestibility of nutrients (Nasri et al., 2011). In addition, sesbania straw contains a moderate amount of anti-nutritional factors such as condensed tannins (Table 2), which may limit the feed intake and reduce the digestibility of protein and structural carbohydrates at higher levels of dietary inclusion (Bekele et al., 2013; Abdullah et al., 2018).

It may be the reduction in the nutrient digestibility of experimental diets on the addition of sesbania straw to the diets or when comparing sesbania straw with alfalfa hay in the medium culture of bacteria and fungi isolated from the rumen was related to the presence of anti-nutritional compounds such as tannins (Tables 1, 2) in sesbania straw. Many researchers have reported the presence of carbohydrates, glycosides, proteins, amino acids, saponins, tannins, alkaloids, phenolic compounds, and flavonoids in sesbania straw (Akram et al., 2021). Tannins have anti-nutritional effects, including reducing digestibility and access to protein, fibers, and digestive disorders in animals, which are mostly the result of disrupting the activity of rumen microorganisms by decreasing nutrient and mineral accessibility, which can result in reduced the digestibility of nutrients (Huang et al., 2018). In addition, tannins lead to the destruction of the cell wall function of rumen microorganisms and interfere with the activity of extracellular enzymes (Besharati et al., 2022). In some cases, the binding of tannin with feed proteins resulted in the lack of nitrogen for rumen bacteria causing a reduction in fiber digestibility. The researchers suggested that tannins prevent microbial digestion by directly inhibiting cellulolytic bacteria and reduce fiber digestion by forming a complex with lignocellulosic compounds (Sharifi et al., 2019). On the other hand, the animals show different reactions and performances to the presence of tannins in the diet, which is caused by the biological reactions of tannins themselves. It is believed that concentration of tannins above 5% in feed and plants can have serious risks for animals (Huang et al., 2018).

According to a study, the use of 63% oak kernels (containing tannins) resulted in a decrease in the population of bacteria, fungi, and ruminal protozoa, as well as the reduction of the disappearance of nutrients and the concentration of fermentation parameters in sheep and goats (Hashemi et al., 2018). Similarly, it was found that incorporating *Calliandra haematocephala* as a tannin source into the diet of the goat reduced the population of fiber-degrading bacteria (Tahmourespour et al., 2017). In another study (Singh et al., 2011), the population of cellulolytic bacteria in animals fed with Pakar (*Ficus infectoria*) leaves (as a rich source of tannin) was significantly reduced compared to the control treatment, which agrees with the results of this experiment. There are many reports regarding the reduction of the digestibility of some nutrients in the diet due to saponin consumption (Kholif, 2023). Therefore, the reduction of nutrient digestibility of the sesbania straw compared to alfalfa hay or the diet containing 100% replacement of sesbania straw with alfalfa hay in the culture medium of fungi and bacteria of the present experiment may be related to these secondary metabolites present in sesbania straw.

On the other hand, diets with 25-75% replacement of sesbania were not effective because the total concentration of anti-nutritional compounds would not have reached a level that could negatively affect the microorganisms and their activity (Farghaly et al., 2022).

In vitro, NH_3-N concentration serves as an indicator of protein degradability because, unlike in the rumen environment, there is no nitrogen uptake or recycling (Hristov et al., 2019). It was reported (Martinez-Fernandez et al., 2020) that the optimal NH_3-N concentration for the growth of rumen microorganisms is 2.75 to 6.3 mg/100 mL, which is consistent with the present study. Probably, the decrease in pH in the diets containing sesbania is due to the presence of some secondary metabolites such as tannin, saponin and so on (Kholif, 2023). It has been reported that tannins have a negative effect on the growth of proteolytic bacteria that are fed for protozoa (Besharati et al., 2022). Therefore, reducing the growth of these bacteria causes a decrease in the rumen protozoa population and, as a result, a decrease in pH. The mentioned results are consistent with some other studies that reported that tannins decrease the pH of rumen fluid (Majewska et al., 2021). Also, it was stated that a decreasing trend was observed in the pH and NH_3-N concentration of the rumen of Holstein calves with the increase in the level of tannin in the processing of soybean meal (Jolazadeh et al., 2015). According to a study feeding sheep tannin-containing feed sources like olive cake and leaves caused a reduction in rumen pH (Oliveira et al., 2023). Similarly, adding 1.2, 1.8, 2.4, and 3.2 grams of saponin to the culture medium (Lila et al., 2003), and feeding the fattening calves with 0, 0.5, and 1% saponin (Lila et al., 2005) resulted in a significant decrease in pH.

The concentration of NH_3-N in tannin-containing diets was 3.7 mg/mL lower than in tannin-free diets, which may be due to the reduction of protein breakdown in the rumen (Orzuna-Orzuna, 2021). Meanwhile, the using oak leaves as a source of tannin in the diet of lambs (Babaei et al., 2015; Tso et al., 2021), green tea and pomace in shrimp (Firdous et al., 2021), and oak kernel powder in sheep (Krueger et al., 2010) did not affect rumen pH and NH_3-N concentration. In agreement with the results of the present experiment, the reduction of NH_3-N concentration due to the use of compounds containing tannins has been stated by other researchers (Rigobello et al., 2023; Tseu et al., 2021; Martello et al., 2020). NH_3-N concentration was lower in tannin-containing diets than in tannin-free diets, which may be due to reduced protein breakdown in the rumen (Besharati et al., 2022). In an experiment, it was stated that one of the reasons for the reduction of NH_3-N concentration at high levels of using the sesbania is probably related to its absorption by the saponin of the sesbania. According to the findings of this research, the consumption of *Yucca gigantea* saponin extract decreased rumen NH_3-N concentration in male calves (Liu et al., 2021) and sheep (Jayanegara et al., 2014). Saponin can bind to ammonia and prevent its excessive increase in the rumen, and when the concentration of rumen NH_3-N decreases, it can be released and help to build microbial protein, of course, saponin in the conditions where ammonia is available sufficiently and constantly. It can play the role of a mediator (Kholif, 2023; Hassan et al., 2020). In other words, saponin is attached to it when the concentration of NH_3-N in the rumen is high and is separated from it when the concentration of NH_3-N in the rumen is low. Therefore, the fluctuation of NH_3-N concentration in the rumen will be such that a sufficient and continuous amount of ammonia is available for microbial production (Hasan et al., 2020). On the other hand, it has been reported that feeding Barbary lambs with 0, 30, 60, and 90 mg of saponin per kg of DM intake, did not affect rumen NH_3-N concentration (Nasri et al., 2011).

Conclusion

Sesbania straw in the ruminant diet at 22.5% (equivalent to 75% replacement in lieu of alfalfa hay) did not have any negative effects on the digestive and fermentation parameters of the ruminal microorganisms. However, when using 30% sesbania straw in the diet (equivalent to 100% replacement for alfalfa hay), there was a decrease in digestive and fermentation activity in some cases. Therefore, the use of the sesbania straw can be recommended in the diet of ruminants, but further experiments on livestock are required to confirm the results.

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