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Assessment of Nutrient Content, *In vitro* True Digestibility, Digestible Protein Content and *In vitro* Fermentation of Rice Straw Ensilaged with Artichoke Leaves

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ABSTRACT

The present study aimed to investigate the nutritional value of silage prepared from rice straw and artichoke leaves. The study consisted of four treatments as follows: T1: only rice straw (RS), T2: 25% artichoke (AC) + 75% RS, T3: 50% AC + 50% RS, and T4: 75% AC + 25% RS. Each treatment group was replicated three times and ensiled for 60 days. The results of the study indicate that the T4 group had significantly lower dry matter (DM) content and neutral detergent fiber (NDF) compared to the other groups ($P<0.05$). Organic matter (OM) and ash content were significantly ($P<0.05$) higher in the T2 group, while crude protein (CP) was significantly ($P<0.05$) higher in the T4 group. *In vitro* true digestibility on dry matter ($IVTD_{DM}$) and on an NDF ($IVTD_{NDF}$) basis were significantly ($P<0.05$) higher in the T4 group. The T4 group exhibited a significantly lower ($P<0.05$) ruminal undegradable protein content and a significantly higher ruminal degradable protein content. *In vitro* total volatile fatty acids (TVFA) were significantly higher ($P<0.002$) in the T4 group, and no significant difference ($P > 0.05$) was among the treatment groups for ammonia nitrogen (NH_3-N) levels and Flieg's score. It can be concluded that rice straw silage prepared with artichoke leaves showed improved nutritional composition and *in vitro* parameters.

Keywords: Artichoke, Fermentation, *In vitro* digestibility, Nutrients, Rice straw, Silage.

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INTRODUCTION

In livestock production, feed costs constitute a substantial 70% of operational expenses. The judicious counsel advocates for a strategic shift toward alternative feed resources, emphasizing the repurposing of kitchen and agricultural waste as viable sources of animal nutrition (Makkar, 2018; Balehegn et al., 2020). Herein lies the untapped potential of rice straw, a colossal agricultural waste, generated globally in the tropics at a staggering annual volume of 800 to 1000 million tons, notably with 600 – 800 million tons emanating from Asian countries (IRRI, 2020). Merely 20% of this abundant resource finds use in industrial applications such as paper and ethanol production, and as fodder. The majority languishes in paddy fields, either left to nourish the soil as compost or succumbing to the flames of field burning (Bahrami et al., 2016). This burning practice unleashes greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O) in the environment (Hong et al., 2021). Instead of burning, utilizing rice straw in the animals' diet is a suitable option. However, rice straw utilization in the diet of ruminants is primarily linked to elevated levels of NDF and ADF, lignification and silicification, along with inadequate crude protein content, resulting in diminished intake, digestibility and palatability (Ravi et al., 2019). One of the primary goals of animal nutritionists is to manipulate the rumen microbial ecosystem to improve the digestion of fibrous feeds, while simultaneously reducing methane emissions and nitrogen waste to enhance the overall productivity of ruminants (Wang, 2025). Enhancing cellulose degradability requires the incorporation of feed additives or unconventional, easily degradable feed resources with high nutritive value. This approach necessitates assessing the

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impact of these additions on ruminal nutrient digestibility (Kazemi, 2025). The concept of associative effects, interactions between different components of a diet that influence the nutritional value of individual ingredients, is particularly important in this context (Sun et al., 2020). These effects can substantially modify microbial fermentation within the rumen, thereby impacting the overall digestibility of feed in ruminants (Robinson et al., 2009; Selim et al., 2024). Previous research has investigated various associative combinations, such as alfalfa and barley straw (Haddad, 2000), legumes and grasses (Dal Pizzol et al., 2017), Kikuyu grass silage and red clover (Guzatti et al., 2017) and corn stalks with alfalfa (Wang et al., 2008). Building on previous findings, the present *in vitro* study, geared toward enhancing the nutritive value of rice straw by ensiling it with agricultural waste from artichoke leaves, thereby offering a promising and innovative approach in optimizing ruminant diets.

Artichoke (*Cynara scolymus L.*) exists in the family Asteraceae, with a global harvest of 1.52 M tonnes in 2020. This vegetable has become a staple in agricultural landscapes, particularly in Türkiye's Aegean, Marmara, and Mediterranean regions, boasting substantial economic profitability (Bektaș and Saner, 2013; Pesce and Mauromicale, 2019; Rana et al., 2023). Nonedible parts of artichoke, including leaves, stems, seed, roots, and outer bracts, can account for up to 80-85% of the plant's total biomass (Zayed and Farag, 2020). Far from mere waste, these byproducts of the artichoke are rich in water-soluble polysaccharides (such as inulin), minerals, vitamins, and phenolic compounds, including flavonoids, anthocyanins, and caffeoylquinic acids (Ahmed et al., 2023). Recent strides in sustainability have led to the utilization of artichoke byproducts in animal forage and the production of biofuel, aiming to reduce waste and management costs (Rana et al., 2023). Fermentative parameter studies are intriguing because they showcase the remarkable ensilage potential of artichoke byproducts, as demonstrated by earlier works (Meneses et al., 2007; Meneses et al., 2020). Notably, these silages proved free of phytosanitary products, rendering them not only harmless but also nutritionally viable as ruminant feeds, presenting a sustainable solution for managing artichoke crop wastes (Ahmed et al., 2023). Previously, silage has been made from the whole stalk of artichoke. To the best of our knowledge and search, no prior studies have explored the realm of co-ensiling rice straw with artichoke leaves. Artichoke leaves were selected for this study due to their softer texture compared to the more fibrous and rigid stems, while the heart or bud is typically reserved for human consumption. This novel approach holds promise for reshaping silage production, as previous studies have highlighted the nutritional value of artichoke leaves (Biel et al., 2020; Ayuso et al., 2024). It was hypothesized that the ensiling of rice straw with artichoke leaves would improve its nutritional value. The objectives of this study were to evaluate the nutrient composition, *in vitro* true digestibility, digestible protein content, and *in vitro* fermentation parameters of rice straw ensiled with artichoke leaves, aiming to assess the feasibility of utilizing agricultural waste as a sustainable feed resource for livestock.

MATERIALS & METHODS

Feed Materials and Experiment Layout

Silage Preparation

To execute this research, Vasco variety of rice straw (RS) and artichoke (AC) leaves were procured from the local agriculture farm of Samsun, Türkiye. Four treatment groups were devised as follows; T1: only rice straw (RS), T2: 25% artichoke (AC) + 75% RS, T3: 50% AC + 50% RS, T4: 75% AC + 25% RS. Each treatment group was replicated three times. To make the silage, rice straw, and artichoke leaves were chopped into 2.5-3cm particle size, and subsequently feed materials were compressed into one-liter glass jars, airtight sealed (after compressing the silage materials in the jars, jars were firstly sealed with polythene material and then were secured with lids) and left for fermentation for 60 days in the dark place. Dry matter was determined by taking two samples from every replicate (jar) of every treatment group. pH was determined by taking 25 grams of silage sample from each of the replicates of every treatment, putting it into a blender machine and adding 100mL of water and then grinding it for homogenization, then liquid homogenate was passed through filter papers into a beaker and measuring pH with pH meter (Mettler Toledo seven compact). pH value of the artichoke leaves' silage was 4.20, and that of fresh rumen fluid was 5.8, while the pH values of the treatment groups are given in Table 1.

Table 1: pH values of treatment groups before and after Daisy[II] analysis

Items	Treatment groups			
	T1	T2	T3	T4
Silage pH	4.80	4.72	4.19	4.24
After Daisy[II] pH	5.42	5.49	5.43	5.47

Nutrient Analysis of Silage and Dried Artichoke Leaves

Following procedures performed nutrient analysis of silage and dried artichoke leaves: The dry matter content of the silage samples and artichoke leaves was determined by drying the samples at 105°C for four hours using a Memmert heating oven ((method 925.09; AOAC, 1995). The ash content was measured by combusting the dried silage samples at 550°C -650°Cfor four hours in a muffle furnace ELF 11/14B, Carbolite, UK) (method 923.05; AOAC, 1995). Crude protein (CP) content was determined using Kjeldahl procedure (method 991.20; AOAC, 1995); nitrogen% % was obtained and multiplied by the 6.25 factor to calculate the CP content. Neutral detergent fiber (NDF) analysis was conducted following the method described by Mertens (2002), utilizing sodium sulfite, an NDF chemical solution, heat-stable alpha-amylase, and a fiber analyzer (Ankom 2002, Ankom Technology Corp., USA). The acid detergent lignin content (11.04%) and chemical composition of the Vasco variety were previously determined in our earlier study (Bölükbaş et al., 2024). The experimental workflow is illustrated in Fig. 1.

Flieg Score Determination

To express the quality of silage, Flieg's score was calculated. The Flieg score was calculated according to this formula: Flieg's Score = 220 + (2 x DM% - 15) - 40 x pH,

Fig. 1: Overview of activities performed during silage preparation and its subsequent analysis.

described by Tatlı Seven et al. (2024). Dry matter and pH of silage were used. The higher the Flieg's score, the better the silage quality. According to the Flieg's scoring system, silage quality is classified as very good (81-100), good (61-80), medium (41-60), low (21-40), and poor (2-1=20). The following scores: a score of 81-100 (very good), 61-80 (good), 41-60 (medium), 21-40 (low), and 0-20 signifies poor quality.

In vitro Parameters of True Digestibility and Protein Determination

In vitro true digestibility analysis was conducted using the ANKOM Daisy^{II} incubator (Ankom Technology Corporation Fairport, NY, USA), following the method described by ANKOM (2002). Rumen fluid was manually collected from the adult sheep slaughtered at a commercial abattoir in the Samsun province of Türkiye. The sheep had been fed a diet with an 80:20 concentrate-to-hay ratio. The collected rumen fluid was immediately transferred to the laboratory in a thermos preheated to 39°C and flushed with 100% pure carbon dioxide to maintain anaerobic conditions and preserve microbial activity (Teng et al., 2024). In the laboratory, the rumen fluid was transferred to the digestion jars by filtering through four layers of cheesecloth.

Fiber filter bags (F57, pore size 40µm) were pre-rinsed with 99.5% acetone for three minutes, dried at 105°C for two hours, and labeled with acid and alkali-resistant pen. Samples were ground (1mm particle size) using a Wiley mill and were weighed (0.5g) into each F57 bag, which was then sealed using a heat sealer, before incubation in rumen fluid.

A buffer solution, consisting of buffer A solution (prepared by adding the following reagents: KH_2PO_4 10g, $MgSO_4.7H_2O$ 0.5g, NaCl 0.5g, $CaCl_2$. $2H_2O$ 0.1g and urea 0.5g, per liter of water) and buffer B solution (prepared by adding Na_2CO_3 15g and $Na_2S.9H_2O$ 1g per liter of water) was prepared following the Ankom Daisy^{II} *in vitro* fermentation system documented in ANKOM (2002).

The digestion procedure was carried out in four digestion jars (vessels), each with a capacity of two litres; Buffer solutions were pre-heated to 39°C in a heating cabinet; 1600mL of buffer solution (1330mL soltion A and about 266mL of soltion B) was poured into each vessel, followed by the addition of 400mL of rumen fluid. Subsequently, six samples (two from each replicate) were placed in each digestion vessel; Incubation was carried out at 39°C for 48 hours using a Daisy^{II} incubator.

After incubation, the digestion fluid was discarded, and the F57 bags were rinsed thoroughly under running water. Neutral detergent fiber (NDF) analysis was then performed using the ANKOM fiber analyzer, following the protocol outlined by ANKOM 2002). Finally, the F57 bags were dried in a forced-air oven at 105°C until a constant weight was achieved. The experimental workflow is illustrated in Fig. 1. The IVTD of all samples was calculated according to the formula described by ANKOM (2002).

$$IVTD\ (\%) = [100 - (W_3 - (W_1 \times C_1) \times 100)] / W_2$$

where:

W_1 represents the tare weight of the empty F57 filter bag,

W_2 is the sample weight,

W_3 denotes the final weight of the filter bag after neutral detergent fiber (NDF) analysis,

C_1 is the blank bag correction, determined as the ratio of the oven-dried weight to the original blank bag weight

Digestible Protein Content

Rumen undegradable protein of treatment groups was determined by putting the two samples from each replicate of every treatment group (a total of 6 samples from each treatment) in DaisyII digestion jars, already having samples for *in vitro* true digestibility without performing acid detergent fiber analysis after the Daisy^{II} procedure. Rumen undegradable protein was calculated using the direct CP values of treatment groups, and after the Daisy^{II} CP values of treatment groups, using the Kjeldahl method. While the rumen degradable protein of the treatment groups was calculated as 100-RUP. Similarly, *in vitro* digestibility, RUP, and RDP of artichoke leaves were determined by using 2 parallel samples from artichoke silage.

Determination of Ammonia-N (NH_3-N) and Total Volatile Fatty Acids

Ammonia nitrogen (NH_3-N), and TVFA were determined using parallel samples from each digestion jar of Daisy[II]. Ten mL of the rumen liquor from each daisy jar was transferred into capped plastic tubes for the determination of volatile fatty acids and NH_3-N. Briefly, a 10mL sample of rumen fluid was placed into a 50 mL beaker, followed by the addition of 4–5 drops of concentrated sulfuric acid (98% H_2SO_4). The mixture was left to stand at room temperature for approximately 2 hours to allow for protein precipitation. It was then centrifuged at 3000 rpm for 10 minutes. From the resulting supernatant, 2mL was carefully transferred to a steam distillation apparatus (Büchi 811 model). Subsequently, 1mL of 40% sodium hydroxide (NaOH) solution was added to release ammonia gas (NH_3). The liberated ammonia was distilled into 5mL of a 2% boric acid solution. A total of 50mL of distillate was collected and titrated with 1/70 N sulfuric acid (H_2SO_4) using a mixed indicator composed of methyl red and bromocresol green. The endpoint was identified by a color change from green to red. The volume of acid used in titration was recorded and used to calculate the concentrations of total volatile fatty acids (TVFA) and ammonia nitrogen (Akyurek and Salman, 2021).

Statistical Analysis

The collected data were subjected to one-way analysis of variance (ANOVA) using SPSS software (version 21; IBM, USA). Differences between means were assessed using Tukey's post hoc test. Results are presented as mean±SEM (standard error of the mean). Before the ANOVA test, the normality and homogeneity of data were tested using the Shapiro-Wilk and Levene's test, respectively. A probability value of $P<0.05$ was considered statistically significant.

RESULTS

Nutrient Composition of Experimental Groups and Artichoke Leaves

The nutritional chemical composition of treatment groups and artichoke leaves on a dry matter basis, as determined through proximate and Ankom analysis procedures, is presented in Table 2 for artichoke leaves and Table 3 for treatment groups, respectively.

Table 2: Chemical composition of artichoke leaves on a dry matter basis

Items	Content %
DM	92.36
OM	84.98
Ash	15.02
CP	10.65
NDF	33.28

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber

It has been observed that there was a significant difference among the different treatment groups with regard to DM, OM, ash, NDF, and CP ($P<0.001$, $P<0.019$, $P< 0.019$, $P<0.001$, and $P<0.034$, respectively). Dry matter results indicate that the T4 group had significantly lower DM than the other treatment groups and control groups. Similarly, OM was significantly higher in the T2 group than in the control group, and significantly higher ash content was observed in the T1 group than in the T2 group and other treatment groups, NDF content was significantly lower in the T4 group than in other treatment groups and control group, and significantly higher CP content was observed in T4 group than other treatment groups.

Table 3: Nutrient composition (%) of treatment groups on DM basis

Parameters	Treatment groups				
	T1	T2	T3	T4	P-value
DM	38.50±0.289^{a}	37.17±0.667^{a}	36.67±0.167^{a}	32.83±0.333^{b}	<0.001
OM	87.45±0.225^{b}	88.39±0.138^{a}	88.07±0.280^{ab}	87.95±0.030^{ab}	0.019
Ash	12.55±0.225^{a}	11.61±0.138^{b}	11.93±0.280^{ab}	12.05±0.030^{ab}	0.019
CP	9.38±0.309^{ab}	9.08±0.127^{b}	9.66±0.072^{ab}	9.86±0.125^{a}	0.034
NDF	69.00±0.349^{a}	65.63±0.773^{b}	62.60±0.133^{c}	56.62±0.295^{d}	<0.001

^{a,b,c,d} Means with different superscripts within the same row differ significantly ($P<0.05$), and results are presented as mean±SEM; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; T1: only rice straw (RS), T2: 25% artichoke (AC) + 75% RS, T3: 50% AC + 50% RS, T4: 75% AC + 25% RS.

Flieg's Score

Flieg's score has been demonstrated in Fig. 2. Fig. 2 presents no significant difference ($P>0.165$) regarding the Flieg's score among different treatment groups.

In vitro Parameters of Digestibility and Protein of Treatment Groups and Artichoke Leaves

In vitro, parameters of digestibility and protein of artichoke leaves and treatment groups have been presented in Table 4 and 5, respectively. As shown in Table 5, there were significant differences ($P<0.001$) among the treatment groups. The T4 group exhibited significantly higher *in vitro* true digestibility of dry matter ($IVTD_{DM}$) compared to the other treatment groups and the control. Similarly, significant differences ($P<0.001$) were observed for the *in vitro* true digestibility of neutral detergent fiber ($IVTD_{NDF}$), with the T4 group showing the highest values among all groups, including the control. Rumen undegradable protein was found significantly ($P<0.05$) lower in the T4 group, followed by the T2 and T3 groups, and lowest in the control group. In contrast, rumen degradable protein was observed significantly ($P<0.05$) higher in the T4 group, followed by the T3 and T2 groups, and the lowest RDP was observed in the control group.

Table 4: *In vitro* parameters determination of artichoke leaves using Daisy[II] incubator

Items	Content %
$IVTD_{DM}$	85.94
$IVTD_{NDF}$	54.09
RUP*	13.70
RDP**	86.30

$IVTD_{DM}$: *in vitro* true digestibility on dry matter basis, $IVTD_{NDF}$: *in vitro* true digestibility on neutral detergent fiber basis; *Rumen undegradable protein after daisy incubation for 48 hours **: Rumen degradable protein after daisy incubation for 48 hours

In vitro Fermentation Parameters of Total Volatile Fatty Acids and Ammonia Nitrogen

The results of the *in vitro* fermentation parameters of the experimental groups are presented in Table 6. The control group exhibited significantly ($P<0.002$) lower total volatile fatty acids (TVFA) concentrations compared to the

Table 5: *In vitro* parameters determination of treatment groups using Daisy[II] incubator

Parameters	Treatment groups				
	T1	T2	T3	T4	P-value
$IVTD_{DM}$	52.36±1.028^{e}	61.24±0.857^{d}	67.23±0.597^{c}	74.65±0.613^{b}	<0.001
$IVTD_{NDF}$	26.76±1.086^{c}	31.77±1.041^{bc}	34.45±0.624^{b}	35.6±0.617b	<0.001
RUP*	51.96±1.019^{a}	47.51±0.823^{b}	44.50±0.774^{b}	37.42±1.895^{c}	<0.001
RDP**	48.04±1.019^{c}	52.49±0.823^{b}	55.50±0.774^{b}	62.58±1.895^{a}	<0.001

$^{a-e}$ Means with different superscripts within the same row differ significantly (P<0.05), and results are presented as mean±SEM; $IVTD_{DM}$: *in vitro* true digestibility on dry matter basis; $IVTD_{NDF}$: *in vitro* true digestibility on neutral detergent fiber basis; *Rumen undegradable protein after daisy incubation for 48 hours **: Rumen degradable protein after daisy incubation for 48 hours; T1: only rice straw (RS), T2: 25% artichoke (AC) + 75% RS, T3: 50% AC + 50% RS, T4: 75% AC + 25% RS.

Table 6: *In vitro* fermentation parameters of treatment groups

Parameters	Treatment groups				
	T1	T2	T3	T4	P-value
TVFA (mmol/L)	64.68±1.075^{c}	69.86±0.875^{ab}	73.20±1.350^{b}	83.53±1.475^{a}	<0.002
NH_3-N (mg/dl)	40.8±8.00	42.40±5.500	42.01±8.500	42.65±3.500	0.348

^{a,b,c} Means with different superscripts within the same row differ significantly (P<0.05), and results are presented as mean±SEM; * TVFA: total volatile fatty caids, NH_3-N: ammonia nitrogen; T1: only rice straw (RS), T2: 25% artichoke (AC) + 75% RS, T3: 50% AC + 50% RS, T4: 75% AC + 25% RS.

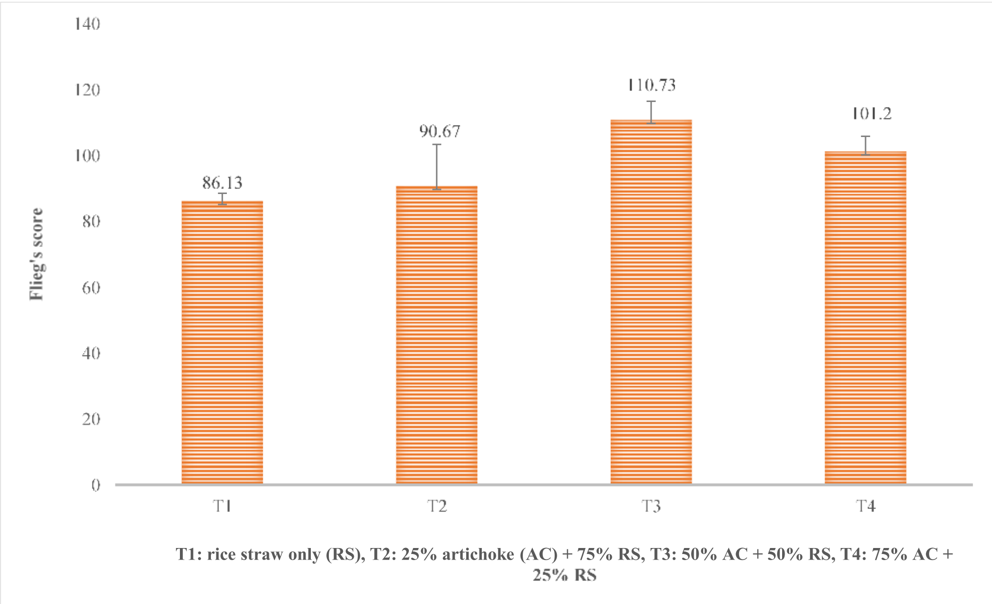

Fig. 2: Flieg's score of treatment groups; T1: only rice straw (RS), T2: 25% artichoke (AC) + 75% RS, T3: 50% AC + 50% RS, T4: 75% AC + 25% RS; and results are presented as mean±SEM; (P>0.05).

treatment groups, with the highest TVFA levels recorded in the T4 group, followed by T3 and T2. In contrast, no significant differences (P>0.05) were observed among the treatment groups regarding ammonia nitrogen (NH_3-N) concentrations.

DISCUSSION

Nutritional Composition of Silage

This study about the rice straw and artichoke leaves silage is one of a kind, and to the best of our knowledge and research, there is limited supportive literature and studies to support and validate our findings. The findings of the present study revealed notable differences in the nutritional composition of rice straw ensiled with artichokes. It was found that DM was significantly (P<0.001) different among different treatment groups. In line with current findings, Özkul et al. (2011) also found higher DM in the silage made from straw, bran, and vegetable waste. Zhao et al. (2019) also found higher dry matter content in the molasses-treated rice straw. Conversely, Lu et al. (2024) found no effect of mixed silage of Chinese cabbage and rice straw on NDF digestibility. The significantly higher OM content in the treatment groups ensiled with artichoke compared to the control group [T2 (88.39%), T3 (88.07%), and T4 (87.95%) vs T1 (87.45%)] indicated that artichoke enhances the preservation of organic matter during ensiling. This finding aligns with previous research by Ahmed et al. (2023), who observed that co-ensiling artichoke bracts with berseem improved silage quality by enhancing rumen fermentation and preserving nutrient content. The control group exhibited significantly higher ash content compared to the artichoke-treated groups. The significantly higher ash content in the control group compared to the treatment groups indicates that the control group contains more mineral content. This difference could also be attributed to the absence of artichoke in the control group, as the dilution effect of artichoke may have contributed to reducing the mineral content in the treatment groups. The other reason for the higher ash content in the control group could be due to the higher silica content found in rice straw (Agbagla-Dohnani et al., 2003). The control group exhibited significantly higher NDF content compared to the artichoke-treated groups. In line with our findings, Chen et al. (2022) found that NDF, ADF, and cellulose contents were decreased significantly in mixed silages (rice straw with citrus pulp, sweet potato peels, and vines) compared to the control. Seifdavati et al. (2021) also found lower NDF content in the silage made of a mixture of vegetable waste, wheat bran, and salt, especially in the group with high vegetable waste percentage. This observation is consistent with the findings of Ahmad et al. (2022), who reported that using biological additives in rice straw silage can lower NDF and acid detergent fiber (ADF) concentrations, leading to improved silage quality. The higher NDF content in the control group (T1:69.00% vs. 56.62% in the T4 artichoke group) further supports this notion. The group ensiled with 75% artichokes had a higher CP content compared to other treatment groups, which is a noteworthy observation. In line with these findings, Ghosh and Islam (2019) reported higher CP content in the rice straw group treated with vegetable waste of cabbage and tomato. The reason behind the high CP content can be the silage technique, as the ensilage process is the most convenient way to conserve the nutritive values of feeds (Khorshed et al., 2006). Contrary to the findings of the present study, Ahmed et al. (2023) found significantly lower CP in the artichoke-treated berseem group. The disparities in CP content of ensiled forages could be attributed to variations in proteolysis of various protein make-ups of ensiled forages due to the associative effect between artichoke and rice straw that may account for the rate and extent of fermentation (Muck et al., 2003). Seifdavati et al. (2021) also found non-significant CP Content in the silage made of a mixture of vegetable waste, wheat bran, and salt (82.5% pumpkin byproducts+17.5% wheat bran plus urea+ probiotics), especially in the group with high vegetable waste percentage.

Flieg's Score

The Flieg's score, calculated from the dry matter content and pH, is used to assess the silages' fermentation quality. The present study showed a non-significant ($P=0.165$) difference among treatment groups regarding the Flieg's score; however, the Flieg's score was numerically higher in treatment groups than in the control group. Similar to these findings, Abo-donia et al. (2021) found that the values of Flieg's Score of rice straw silage were significantly ($P<0.05$) improved when rice straw was treated with whey or molasses plus urea. Similarly, D'Alessandro et al. (2023) reported a Flieg's score exceeding 100 in silages formulated with a mixture of grape pomace, wheat straw, olive mill wastewater, and cheese whey. A Flieg's score of 100 indicates superior silage quality, as noted by Dong et al. (2017). In the current study, all silages achieved the Flieg's score above 80, suggesting good quality silage in line with the classification proposed by Liu et al. (2024).

***In vitro* Digestibility**

Studies on digestibility have become widely accepted as a means of assessing the nutritional value and intake of feed (Huhtanen et al., 2007). The results of the $IVTD_{DM}$ and $IVTD_{NDF}$ are crucial indicators of the nutritive value of the ensiled materials. In the present study, significant differences ($P<0.001$) were observed among the treatment groups in both $IVTD_{DM}$ and $IVTD_{NDF}$, highlighting the impact of ensiling with varying proportions of artichoke. Notably, the group ensiled with 75% artichoke exhibited the highest $IVTD_{DM}$ and $IVTD_{NDF}$ values as compared to the control group, indicating that the inclusion of artichoke at this level significantly enhances the digestibility of the silage. In line with the findings of the current study, Du et al. (2022) found in their study that paper mulberry ensiling with rice straw hay (10%) resulted in better $IVTD_{DM}$ compared to 20% and 30% of rice straw hay. Sun et al. (2020) reported that true digestibility of dry matter (TDMD) increased when corn straw was co-fermented with timothy hay and corn grain. Seifdavati et al. (2021) also found higher $IVTD_{DM}$ in the silage made of a mixture of vegetable waste, wheat bran, and salt (82.5% pumpkin byproducts+17.5% wheat bran+urea+ probiotics), especially in the group with high vegetable waste percentage. Zhao et al. (2019) found higher $IVTD_{DM}$ and neutral detergent fiber in the molasses-treated rice straw. In contrast to the present study, Lu et al. (2024) reported no significant differences in NDF digestibility and ADF digestibility of Hu sheep between the control group and the group fed mixed silage of cabbage waste and rice straw ($P>0.05$). The improvement in $IVTD_{DM}$ can be attributed to the associative effects between fermented rice straw and artichoke leaves, as well as the higher organic matter and lower neutral detergent fiber (NDF) content in the mixed feed treatments. These characteristics provided more fermentable carbohydrates to rumen microorganisms. Associative effects occur when the digestibility of a feed mixture differs from the sum of its individual components. Beneficial associative effects are often observed when low-quality forages are combined with feeds rich in nitrogen, easily digestible carbohydrates, and low fiber content. Several studies—both *in vitro* and *in vivo*—have shown that supplementing rice straw with nitrogen-rich feedstuffs enhances its digestibility (Zhang et al., 2010; Tagliapietra et al., 2014; Fattah et al., 2020). Digestibility variations are primarily influenced by the chemical composition of the feed, especially the levels of lipids, pectin, and NDF, which significantly impact *in vitro* fermentation characteristics (Meneses et al., 2020). The observed improvements in $IVTD_{DM}$ and *in vitro* true digestibility of NDF ($IVTD_{NDF}$) may also be due to reduced dry matter (DM) loss in the artichoke leaf-treated groups, as easily digestible components such as water-soluble contents and crude protein (CP) remained intact prior to incubation (Zhao et al., 2019). Improvement in $IVTD_{NDF}$ can be explained by the findings of previous research, stating that higher content of hemicellulose can be digested more easily than cellulose, primarily because of its amorphous structure and the lesser degree of polymerization and availability of other soluble fiber contents (Zhao et al., 2019; Zeaiter et al., 2019). The inclusion of artichoke in the present study supports previous findings that ensiled vegetable co-products can sustain high *in vitro* dry matter disappearance. Notably, the group receiving 75% artichoke showed a significant increase in both $IVTD_{DM}$ and $IVTD_{NDF}$, indicating improved overall dry matter and fiber digestibility. This suggests that artichoke is a highly digestible feed component for ruminants, likely due to its structural properties that enhance microbial breakdown in the rumen (Costabile et al., 2010; García-Rodríguez et al., 2019).

In vitro Fermentation

In vitro fermentation parameters, specifically, the production of total volatile fatty acids (TVFA) and ammonia (NH_3-N), are essential indicators of rumen fermentation efficiency and microbial activity (Li et al., 2022). In the present study, significantly higher TVFA production was observed in the T4 treatment groups compared to the control group. Similar to the outcomes of the current study, Sun et al. (2020) reported that TVFA increased when rice straw was co-fermented with timothy hay and corn grain. Rymer and Givens (1999) also reported that roughages with high fermentable carbohydrates (molasses, wheat, sugar beet feed, grass silage, and maize) released larger amounts of gas and VFA. The results predict that ensiling rice straw and artichoke together increases NFCs from artichoke and the easily fermentable hemicellulose, cellulose, and other soluble components, which influence the rumen microorganisms and fermentation environment, producing more TVFA (Sun et al., 2020; Fissore et al., 2024). In the present study, there was a non-significant difference among treatment groups concerning NH_3-N production. However, the numerically high value of NH_3-N was observed in the T4 group (75% artichoke). Ahmed et al. (2023) reported higher ammonia-N concentration in higher artichoke bract content at 50% and 100% artichoke levels (27.00mg/100mL; 25.36mg/100mL vs 23.52mg/100mL control). This increase in ammonia content can be linked to the high content of soluble proteins and non-protein nitrogenous substances present in the artichoke leaves and the increase in the ensiling time (Ahmed et al., 2023; Rosani et al., 2025). A study conducted by Du et al. (2022) reported that paper mulberry ensiling with rice straw hay (10%) resulted in lower NH_3-N compared to 20% and 30% of rice straw hay.

Conclusion

The present study demonstrated that the nutritional composition and *in vitro* true digestibility of silage have been considerably impacted by the combination of agricultural wastes of artichoke leaves and rice straw. The findings showed that increasing the artichoke content in the silage improved several key nutritional factors. The group containing 75% artichokes (T4) had significantly the lowest neutral detergent fiber (NDF) and the highest crude protein content. Additionally, the T4 group had considerably higher *in vitro* true digestibility (IVTD) on a dry matter basis and NDF basis, indicating improved feed utilization. Low ruminal undegradable protein and higher digestible protein contents were found in the T4 group. Group T4 had higher total volatile fatty acids. Overall, the study highlights the potential of combining artichoke leaves with rice straw to enhance silage quality, particularly at higher inclusion levels (up to 75%), and supports their use as a sustainable feed resource for livestock. For future researchers and practical application by farmers, it is recommended to investigate the inclusion of various levels and parts of artichoke in silage preparations alongside other agricultural by-products such as wheat straw, corn cobs, corn straw, sugarcane bagasse, and grain wastes.

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