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EFFECT OF UREA AND/OR CRUDE GLYCERIN TREATMENT ON THE NUTRIENT COMPOSITION AND *IN VITRO* TRUE DIGESTIBILITY OF DIFFERENT VARIETIES OF RICE STRAW

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ABSTRACT

This study aimed to evaluate the nutrient composition and *in vitro* true digestibility of three rice straw (RS) varieties with varying stem lengths (Vasco (V): short-stemmed, Cammeo (C): medium-stemmed, and Efe (E): long-stemmed) treated with urea and/or crude glycerin. Each RS variety was ensiled in laboratory-scale mini-silos for 30 days using the following treatments: i) Control, ii) 5 % urea (U) treated RS, iii) 5 % crude glycerin (G) treated RS, and iv) 5% urea + 5% crude glycerin (UG) treatment RS. The Vasco variety of RS exhibited superior nutritional value compared to the Cameo and Efe varieties, with a greater ($P<0.05$) *in vitro* true digestibility of dry matter ($IVTD_{DM}$ (V:46.11%, C:41.17% and E:43.94%) as well as lower ($P\leq0.001$) NDF (V:66.38%, C:71.68%, and E:68.59%) and ADF (V:43.61%, C:50.64%, and E:49.1%) levels. Silage of all cultivars demonstrated comparable responses to the applied treatments. The crude protein ($P<0.001$) and $IVTD_{DM}$ ($P<0.001$) were greater in all treatment groups where urea was used alone or in combination with crude glycerin. All cultivars showed a reduction in NDF ($P<0.001$) and ADF ($P<0.001$) levels with the application of crude glycerin treatment alone, but no significant effect was observed on $IVTD_{DM}$ values. In the interaction effect, Efe×UG showed higher ($P<0.001$) *in vitro* true digestibility while for *in vitro* NDF digestibility, Efe×U interaction exhibited higher ($P<0.001$) digestibility compared to other interaction effects. The findings of the study suggest that adding urea or a combination of urea and glycerin to rice straw improved its nutritional content and digestibility. Treating rice straw with a combination of urea (5%) and glycerin (5%) could be a promising strategy to improve its nutritional value for livestock feed.

Keywords: crude glycerin, digestibility, rice straw, silage, treatment, urea

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INTRODUCTION

Rice straw is the largest annual crop residue produced globally as a by-product of rice harvesting (Zhao *et al*., 2019). Rice straw is widely used in ruminant feeding in Asian countries where rice cultivation is common. However, the high level of silicification and lignification, slow ruminal degradation, and low nutrient content are the main limitations of rice straw and restrict its use as a ruminant feed (Van Soest, 2006). Therefore, the majority of the straw is either left unutilized or burned directly, leading to the waste of resources and environmental pollution (Bölükbaş and Kaya, 2018).

To optimize the utilization of rice straw as a ruminant feed, several physical, chemical, and activity-based treatment methods, including supplementation with other feed materials or components have been explored (Sarnklong *et al*., 2010; Abo-Donia *et al*., 2021). Among these, urea treatment is the most widely used method because of its practical applicability and non-hazardous nature (Sarnklong *et al*., 2010). Urea can serve as both a nitrogen source and a delignification agent, which can break the ester bonds present between the cellulose, hemicellulose, and lignin structures of the rice straw. These processes increase the degradability of the treated straw by allowing rumen microorganisms to attack structural carbohydrates more effectively (Wanapat *et al*., 2009). However, treating straws with urea alone may result in reduced voluntary feed intake due to the excessive ammonium odor and low fermentation efficiency. Therefore, urea is often applied in combination with energy sources such as molasses to reduce the strong ammonium odor, increase the palatability of the diet, and better utilization of ammonical nitrogen in the treatment process (Sheikh *et al.,* 2017). Crude glycerin (CG) is a by-product of the biodiesel industry and mostly consists of glycerol (Bölükbaş and Kaya, 2022). The production of one liter of biodiesel results in the generation of 0.1 liters of crude glycerin. In 2020, global biodiesel production was reported to be 40 billion liters and is expected to reach 46 billion liters by 2029, according to OECD-FAO (2020). The expansion of biodiesel production has led to a significant increase in the amount of crude glycerin, which could potentially provide a more cost-effective source of energy compared to conventional sources like molasses. Due to the presence of glycerol in crude glycerin, it has the potential to be used as an additive to improve silage fermentation. Glycerol serves as a highly bioavailable energy substrate that promotes the growth of anaerobic microorganisms, particularly lactic acid bacteria, thereby enhancing fermentation efficiency and the nutritive quality and stability of the ensiled roughages. A limited number of studies have reported the positive effects of crude glycerin on silage fermentation, such as in sugarcane silage (Santos *et al.,* 2015) and cassava leaves silage (Syahniar *et al.,* 2018). However, to the best of our knowledge, there is no prior research on the utilization of crude glycerin in the treatment of rice straw.

There are over 40,000 rice varieties cultivated in the world (TRA, 2020). Depending on the environmental conditions, straws of these varieties may differ in terms of their morphology, nutrient content, and digestibility (Wahyono *et al.,* 2021). Plant breeding efforts have recently focused on developing short rice varieties with higher grain yields. High-yielding short varieties tend to have more digestible straws (Shahjahan *et al.,* 1993). On the other hand, tall varieties tend to be more leafy (Vadiveloo, 1995), and unlike other cereal straws, the leaf portions of rice straw have less digestibility than the stems (Van Soest, 2006). Vadiveloo (2003) reported that urea treatments were proved to be more effective in increasing *in vitro* dry matter degradability of rice cultivars with low degradability than those with high degradability (45% vs 55-62%). Many rice varieties are being cultivated in Türkiye. However, there is scarce information about the nutrient composition, digestibility, and treatment response of the straws of these cultivars.

The nutrient content and digestibility of rice straws are important factors for determining their suitability as livestock feed. However, straws from rice varieties with different plant heights may exhibit variations in these parameters and may also respond differently to chemical treatments. Therefore, in this study, we aimed to investigate the effect of treatment with urea and/or crude glycerin on the nutrient composition and *in vitro,* true digestibility of short-stemmed (Vasco), medium-stemmed (Cammeo) and tall-stemmed (Efe) rice varieties widely cultivated in Turkey, in order to provide insights into their potential use as a low-cost feed alternative.

MATERIALS AND METHODS

The preset study was conducted in the Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun Province, Turkiye. The rumen samples were collected from slaughtered animals, thus approval from the ethics committee was not deemed necessary.

The treatment process of rice straw varieties: Three varieties of rice straw were used in this study, consisting of two Italian varieties (Vasco and Cammeo) and one Turkish-registered variety (Efe). Rice straw (RS) was collected after harvesting in October 2021 from various local paddy cultivation fields in the Bafra district of Samsun province, located in the Kızılırmak Delta, Turkiye. The crude glycerin was acquired from biodiesel production based on sunflower oil and was bought from Aydinlar Kimya (Istanbul, Turkiye), and contained 85.44% glycerol, 9.63% moisture, 3.11% salt, and <0.009% methanol. The urea was supplied from Isolab (Wertheim, Germany). The straws were chopped into lengths of 3 to 4 cm with a fodder chopper, and then ensiled using the following treatments: i) Control untreated (two litres distilled water was added to 1 kg fresh rice straw) ii.) 5% Urea treatment (1 kg of fresh rice straw was treated with 50-gram urea and then macerated with two litres of distilled water to increase the moisture content) iii) 5% Crude glycerin treatment (1 kg of fresh rice straw was treated with 50 ml of crude glycerine (purity 85%) and then macerated with two litres distilled water) iv) 5% urea + 5% crude glycerin treatment (1 kg of fresh rice straw was treated with 50 g urea and 50 ml crude glycerine and then macerated with two litres distilled water). The rates of urea application used in this study were based on previous studies (Vadiveloo, 2003; Vadiveloo and Fadel, 2009).

The treated rice straw consisted of four replicates of each treatment for each variety (a total of 48) and was placed into one-liter laboratory glass bottles. These bottles were sealed with screw tops and plastic tape to create an anaerobic environment in the bottles and then stored at room temperature (22°C to 28°C) for 30 days. After 30 days of incubation, the silos were opened and dried in a forced-air oven (UNB 100, Memmert, Germany) at 60 °C for 48 h. Then, the rice straw was ground by using a grinding Wiley mill to a size of 1mm and then used for the analysis of nutrient composition and the *in vitro* assays.

Chemical analysis: The dry matter (DM) content was determined by drying the samples at 105°C for 4 hours (AOAC, 1995; method 925.09). The content of ash in the silage samples of rice straw was estimated by combustion of the samples in a muffle furnace (ELF 11/14B, Carbolite, UK) (AOAC, 1995; method 923.03) for 4 hours at 550°C. The crude protein was determined using the Kjeldahl procedure (AOAC, 1995; method 991.20).

The ether extract (EE) content was determined using a Soxhlet extractor (B-811, BUCHI, Switzerland) (AOAC, 1995; method 920.29). The estimation of neutral detergent fiber (NDF) was performed by applying the method described by Mertens (2002), using NDF solution, triethylene glycol, sodium sulfite and heat-stable alpha-amylase. The acid detergent fiber (ADF) was determined according to AOAC (1995; method 973.18), using cetyl trimethylammonium bromide and 1N sulfuric acid. To determine the both neutral detergent fiber (NDF) and acid detergent fiber (ADF), a laboratory instrument, namely a Fiber Analyzer (Ankom 200, Ankom Technology Corp., USA) was used, and the results were expressed inclusively as residual ash. Acid detergent lignin (ADL) was determined by extraction of the ADF fraction with 72% sulfuric acid (Van Soest, 1963).

***In vitro* true digestibility:** The ANKOM Daisy^{II} Incubator (ANKOM Technology Corporation) was employed to perform *in vitro* true digestibility (IVTD) analysis, following the procedure specified in ANKOM (2002). The rumen fluid was obtained from the rumens of two six-year-old Holstein cattle slaughtered at a commercial abattoir in Samsun, Turkey. The cattle were fed a diet consisting of grass hay and concentrate with a ratio of 65:35, respectively. The rumen fluid was collected manually post-slaughter in a thermos that was preheated to 39°C and had CO_2, and then immediately transported to the laboratory. In the laboratory, filtration of the rumen fluid was performed using four layers of cheesecloth. The fiber filter F57 bags (with a pore size of 40 µm) were rinsed with 99.5% acetone for three minutes, dried in a forced-air oven for two hours at 105ºC, and marked with acid-alkaline resistant pens. The ground samples of 1 mm were weighed 0.5 g in each F57 filter bag. The filter bags were sealed with a heat sealer before incubation in rumen fluid. A buffer solution was prepared according to the procedure of Ankom Daisy^{II} *in vitro* fermentation system outlined in ANKOM (2002). The analysis was carried out in four digestion jars, each jar with a capacity of two litres of solution. A volume of 1.6 L buffer solution, heated to 39 °C, was poured into each digestion jar. Then a volume of four hundred (400) mL of rumen fluid was poured into each jar. Each unit contained 24 samples, with 4 replicates of each straw variety. The samples were incubated for 48 hours at 39 °C. After the incubation period was completed, the liquid in the digestion jars was discarded and filter bags were rinsed under running water. After that, the neutral detergent fiber analysis was performed using the Ankom Fiber Analyzer, as per the method outlined in ANKOM (2002). The fiber filter bags were kept in the forced-air oven until they achieved a constant weight at 105 °C. The values of IVTD of all samples were calculated using the formula described in ANKOM (2002);

$$IVTD (\%) = [100 – (W_3 – (W_1 \times C_1) \times 100)] / W2$$

where:
W_1: Tare weight of empty F57 filter bag
W_2: Sample weight
W_3: Final bag weight after NDF analysis
C_1: Blank bag correction (oven-dried weight/original blank bag weight)

Statistical analysis: The data were analyzed using SPSS 14.01 software (IBM, USA). To minimize the risk of type I error (alpha = 0.05) and to achieve a power of 0.90 for the study, 4-5 jars per group were determined as the appropriate sample size using the PS Sample Size and Power Calculator from Vanderbilt University, TN. All the results were reported as mean ± SEM (standard error of the mean). Before conducting the statistical analysis, the normality and homogeneity of data were assessed using the Shapiro-Wilk and Levene's tests, respectively. One-way ANOVA was used to analyze the main effects and Tukey's test was applied to compare the means, while interaction effects were analyzed by GLM, using pairwise comparisons for interaction factors, and the Bonferroni post-hoc test was used to compare the multiple pairs of means (Field, 2018)

RESULTS

Nutrient composition and *in vitro* true digestibility of rice straw varieties without treatment: Nutrient compositions and *in vitro true* digestibility (IVTD) of the untreated rice straw varieties are presented in Table 1. The highest ash content and the lowest crude protein (CP) content were found in the Efe variety (P<0.001). The lowest ash and highest CP were observed in the Vasco variety (P<0.001). The NDF and ADF contents of the Vasco variety were lower than the Cameo and Efe varieties (P≤0.001). There was no difference in the ADL content among all varieties (P=0.835). The highest metabolizable energy (ME) value was calculated in the Vasco cultivar straw (P<0.001).

The lowest $IVTD_{DM}$ value among the straws was noted in the Cameo variety (P<0.05), while the values of $IVTD_{DM}$ of the Vasco and Efe varieties were similar. There was no significant difference in the $IVTD_{NDF}$ values among all the rice straw varieties (P=0.054).

Nutrient profile and *in vitro* true digestibility of urea and/or crude glycerin treat- rice straw varieties

Treatment of Vasco cultivar straw: The nutrient composition and IVTD of the Vasco variety treated with urea and/or crude glycerin have been demonstrated in Table 2. The groups treated with urea (U and UG) showed a significant increase in CP content compared to the untreated control group (P<0.001). Moreover, significantly lower NDF and ADF levels of the groups treated with crude glycerin (G and UG) were observed than those of the U and control groups (P<0.001). Urea treatment did not affect the metabolizable energy, while crude glycerin treatment led to higher ME values (P<0.001).

Crude glycerin treatment did not affect the $IVTD_{DM}$ value of the Vasco variety. However, the groups treated with urea alone and with glycerin together showed an increase in $IVTD_{DM}$ (P<0.01). Among the treatment groups, the highest $IVTD_{NDF}$ level was observed in the group treated with urea, while the lowest $IVTD_{NDF}$ level was observed in the group treated with crude glycerin.

Table 1. Nutrient composition and *in vitro true* digestibilities of non-treated rice straw varieties

Items, %	Rice straw varieties			P-value
	Vasco	Cameo	Efe	
Dry matter	94.98±0,52	96.29±0,58	96.19±0,27	0.152
Ash	10.64±0.03[c]	13.88±0.27[a]	14.97±0.05[b]	<0.001
Organic matter	85.34±0.49[a]	82.40±0.32[b]	80.19±0.31[c]	<0.001
Crude protein	9.14±0.26[a]	4.08±0.06[b]	3.80±0.06[c]	<0.001
NDF	66.38±0.26[b]	71.68±0.76[a]	68.59±0.78[a]	≤0.001
ADF	43.61±0.41[b]	50.64±0.80[a]	49.1±0.17[a]	<0.001
ADL	11.04±0.36	10.83±0.32	11.15±0.41	0.835
[1]ME, Mcal kg^{-1}	1.62±0.01[a]	1.19±0.03[b]	1.05±0.01[b]	<0.001
		Digestibility, %		
[2]$IVTD_{DM}$	46.11±1.30[a]	41.17±1.52[b]	43.94±1.21[b]	<0.05
[3]$IVTD_{NDF}$	21.21±0.95	20.09±0.43	22.15±0.91	0.054

[a,b,c] Within a row means with different superscripts differ significantly (P< 0.05).
[1]ME: metabolisable energy; [2]$IVTD_{DM}$: *in vitro* true digestibility on dry matter basis; [3]$IVTD_{NDF}$: *in vitro* true digestibility on neutral detergent fiber basis

Table 2. The nutrient composition and *in vitro true* digestibilities of Vasco rice straw after treatment with urea and/or crude glycerin

Items, %	Treatments				P-value
	[1]Control	[2]U	[3]G	[4]UG	
Dry matter	32.85±0.54[b]	32.38±0.2[b]	35.46±0.48[a]	34.03±0.49[ab]	<0.05
Ash	10.76±0.14	10.69±0.07	10.44±0.1	10.69±0.36	0.69
Organic matter	83.53±0.1[ab]	82.52±0.26[b]	83.65±0.35[ab]	84.26±0.42[a]	<0.05
Crude protein	9.11±0.09[c]	10.87±0.37[ba]	9.84±0.25[bc]	11.19±0.22[a]	<0.001
NDF	71.73±0.78[a]	70.66±1.08[a]	64.91±1.2[b]	65.64±0.32[b]	<0.001
ADF	47.91±0.38[a]	49.12±0.71[a]	43.07±0.72[b]	44.12±0.58[b]	<0.001
ADL	13.13±0.48[ab]	13.86±0.16[a]	12.9±0.15[ab]	12.33±0.18[b]	<0.05
ME[5], Mcal kg^{-1}	1.43±0.01[ab]	1.45±0.03[b]	1.64±0.03[a]	1.64±0.03[a]	<0.001
Digestibility, %					
[6]$IVTD_{DM}$	47.86±1.8[b]	57.06±2.13[a]	47.55±2.8[b]	57.77±0.66[a]	<0.01
[7]$IVTD_{NDF}$	25.87±0.98[c]	35.24±0.71[a]	20.82±0.91[d]	30.16±0.71[b]	<0.01

[a, b, c] Within a row means with different superscripts differ significantly (P < 0.05).
[1]Control: ensiled Vasco rice straw variety treated with water; [2]U: ensiled Vasco variety treated with %5 urea; [3]G: ensiled Vasco Vasco variety treated with %5 crude glycerin; [4]UG: ensiled Vasco variety treated with %5 urea and %5 crude glycerin;[5]ME: metabolisable energy; [6]$IVTD_{DM}$: *in vitro* true digestibility on dry matter basis; [7]$IVTD_{NDF}$: *in vitro* true digestibility on neutral detergent fiber basis

Treatment of Cammeo cultivar straw: The nutrient composition and IVTD of the Cameo variety treated with urea and/or crude glycerin are presented in Table 3. An increase in CP level was observed in the U and UG groups treated with urea (P<0.001). The NDF and ADF levels of all treatment groups were found lower than those of the control group (P<0.001). The lowest ADF and NDF levels were obtained in the treatment group where urea and crude glycerin were used together (P<0.001). Urea treatment did not affect the metabolizable energy, while higher ME values were obtained in the rice straw treated with crude glycerin (<0.001).

The $IVTD_{DM}$ of all treatment groups was higher than that of the control group (P<0.001). The highest $IVTD_{DM}$ value was observed in the UG group. Among the treatment groups, the highest $IVTD_{NDF}$ level was observed in the group treated with urea, while the lowest level of $IVTD_{NDF}$ was found in the group treated solely with glycerin (G).

Table 3. The nutrient composition and *in vitro* true digestibilities of Cameo rice straw after treatment with urea and/or crude glycerin.

Items, %	Treatments				P-value
	[1]Control	[2]U	[3]G	[4]UG	
DM	31.24±0.24[b]	32.91±0.18[b]	34.43±0.4[a]	34.76±0.36[a]	<0.001
Ash	13.89±0.37	13.44±0.4	13.16±0.29	12.78±0.35	0.212
Organic matter	80.18±0.45[b]	79.68±0.46[b]	81.18±0.52[ab]	82.56±0.27[a]	<0.05
Crude protein	4.06±0.05[b]	7.57±0.18[a]	4.63±0.06[b]	6.21±0.15[c]	<0.001
NDF	74.94±0.73[a]	71.94±0.3[b]	68.67±0.87[c]	66.96±0.3[c]	<0.001
ADF	53.92±0.75[a]	50.99±0.12[b]	49.23±0.55[b]	49.03±0.33[b]	<0.001
ADL	13.19±0.59[a]	12.1±0.19[ab]	11.79±0.28[ab]	10.96±0.41[b]	<0.05
[5]ME, Mcal kg^{-1}	0.93±0.04[b]	1.03±0.01[b]	1.15±0.02[a]	1.15±0.02[a]	<0.001
Digestibility, %					
[6]$IVTD_{DM}$	43.88±1.99[b]	56.52±1.35[a]	46.22±2.67[b]	57.83±2.97[a]	<0.001
[7]$IVTD_{NDF}$	24.54±0.6[b]	36.21±0.74[a]	22.01±0.92[b]	31.06±1.09[c]	<0.001

[a, b, c] Within a row means with different superscripts differ significantly ($P < 0.05$).
[1]Control: ensiled Cameo rice straw variety treated with water; [2]U: ensiled Cameo variety treated with %5 urea; [3]G: ensiled Cameo variety treated with %5 crude glycerin; [4]UG: ensiled Cameo variety treated with %5 urea and %5 crude glycerin;[5] metabolisable energy; [6]$IVTD_{DM}$: *in vitro* true digestibility on dry matter basis; [7]$IVTD_{NDF}$: *in vitro* true digestibility on neutral detergent fiber basis

Treatment of Efe cult var straw: The nutrient composition and IVTD of the Efe rice straw variety treated with urea and/or glycerin are shown in Table 4. Significantly higher CP levels were observed in the U and UG groups treated with urea compared to the untreated control and G groups (P<0.001). The UG group treated with urea and crude glycerin exhibited the lowest NDF and ADF levels among all groups (P<0.001). The NDF and ADF levels of the U group treated with urea alone were found to be comparable to those of the control group. The use of crude glycerin alone and in combination with urea in the G and UG groups led to a significant reduction in NDF and ADF levels (P<0.001). The ME levels of all groups treated with urea and/or glycerin were higher than the control group (P<0.001).

The $IVTD_{DM}$ of the U and UG groups was higher than that of the control group (P<0.001). Only the G group treated with crude glycerin had an $IVTD_{DM}$ value similar to that of the control group. Notably, the UG group exhibited the highest $IVTD_{DM}$ value (P<0.001). Additionally, the $IVTD_{NDF}$ value of the treatment groups treated with U alone and in combination with glycerin was found to be significantly higher than that of the C and G groups (P<0.001).

Table 4. The nutrient composition and *in vitro* digestibilities of Efe rice straw after treatment with urea and/or crude glycerin.

Items, %	Treatments				P-value
	[1]Control	[2]U	[3]G	[4]UG	
DM	30.82±0.3[b]	32.9±0.37[a]	33.63±0.39[a]	34.3±0.28[a]	<0.001
Ash	14.91±0.25	13.98±0.28	14.09±1.24	13.42±0.24	0.092
Organic matter	78.14±0.27[a]	79.88±0.53[ab]	78.87±0.84[b]	81.39±0.14[a]	<0.05
Crude protein	3.42±0.07[b]	5.83±0.38[a]	3.75±0.15[b]	5.5±0.1[a]	<0.001
NDF	72.58±0.51[a]	71.83±0.31[ba]	69.83±0.7[b]	67.04±0.58[c]	<0.001
ADF	56.78±0.72[a]	54.42±0.48[ab]	51.57±0.46[b]	50.77±0.42[b]	<0.001
ADL	12.76±0.39	12.04±0.33	12.56±0.22	12.87±0.32	0.31
ME, Mcal kg^{-1}	0.84±0.02[c]	0.93±0.03[b]	1.00±0.06[ba]	1.11±0.02[a]	<0.001
Digestibility, %					
$IVTD_{DM}$	45.98±1.88[b]	57.38±2.3[a]	48.55±1.82[b]	60.08±1.95[a]	<0.001
$IVTD_{NDF}$	27.15±0.78[b]	35.83±0.61[a]	26.66±0.64[b]	33.13±0.93[a]	<0.001

[a, b, c] Within a row means with different superscripts differ significantly ($P < 0.05$).
[1]Control: ensiled Efe rice straw variety treated with water; [2]U: ensiled Efe variety treated with %5 urea; [3]G: ensiled Efe variety treated with %5 crude glycerin; [4]UG: ensiled Efe variety treated with %5 urea and %5 crude glycerin;[5] metabolisable energy; [6]$IVTD_{DM}$: *in vitro* true digestibility on dry dry matter basis; [7]$IVTD_{NDF}$: *in vitro* true digestibility on neutral detergent fiber basis.

Interaction effect of rice straw varieties and chemical treatments on $IVTD_{DM}$ and $IVTD_{NDF}$ digestibility: The interaction effect of different rice straw varieties and chemical treatments on *n v tro* true digestibility on a dry matter basis has been shown in Table 5. The interaction effect of Efe and urea+crude glycerin showed significantly higher (P<0.001) *in vitro* true digestibility ($IVTD_{DM}$) as compared to the rest of the interaction effects with Cameo and control interaction having the least *in vitro* true digestibility value.

Table 6 illustrates the interaction effect of rice straw varieties and chemical treatments on the *in vitro* true neutral detergent fiber digestibility ($IVTD_{NDF}$). Efe and urea showed significantly greater (P<0.001) *in vitro* NDF digestibility followed be Vasco× urea , Cameo× UG, Vasco×control, Cameo×control with least effect showed by Vasco and crude glycerin.

Table 5. Interaction effect of rice straw varieties and chemical treatments on the *in vitro* true digestibility ($IVTD_{DM}$).

Variety treatment	[1]Control	[2]U	[3]G	[4]UG	P-value
Vasco	48.72±0.693[d]	55.54±0.693[c]	46.66±0.693[e]	57.36±0.693[bc]	<0.001
Cameo	40.52±0.693[f]	56.45±0.693[bc]	48.07±0.693[de]	59.8±0.693[a]	
Efe	48.62±0.693[d]	57.57±0.693[b]	49.99±0.693[d]	60.58±0.693[a]	

[a-f] Within a row means with different superscripts differ significantly (P< 0.05)

$IVTD_{DM}$: the *in vitro* true digestibility on dry matter basis; [1]Control: ensiled rice straw varities treated with water; [2]U: ensiled rice straw varities treated with %5 urea; [3]G: ensiled rice straw varieties treated with %5 crude glycerin; [4]UG: ensiled rice straw varieties treated with %5 urea and %5 crude glycerin

Table 6. Interaction effect of rice straw varieties and chemical treatments on the $IVTD_{NDF}$ digestibility

Variety treatment	[1]Control	[2]U	[3]G	[4]UG	P-value
Vasco	23.46±0.867[e]	31.32±0.867[b]	17.46±0.867[g]	26.35±0.867[de]	<0.001
Cameo	20.18±0.867[f]	32.58±0.867[ab]	20.86±0.867[f]	30.03±0.867[c]	
Efe	25.8±0.867[de]	33.57±0.867[a]	25.1±0.867[de]	31.3±0.867[abc]	

[a-g] Within a row means with different superscripts differ significantly (P < 0.05)

$IVTD_{NDF}$: the *in vitro* true neutral detergent fiber digestibility on a dry matter basis; [1]Control: ensiled rice straw varieties treated with water; [2]U: ensiled rice straw varieties treated with %5 urea; [3]G: ensiled rice straw varieties treated with %5 crude glycerin; [4]UG: ensiled rice straw varieties treated with %5 urea and %5 crude glycerin

DISCUSSION

Nutrient composition and *in vitro* true digestibility of rice straw varieties without treatment: The CP values of the straws belonging to the Efe and Cameo varieties were found to be consistent with the CP values reported in many studies (Syahniar *et al.,* 2018; Ma *et al.,* 2020; Abo-Donia *et al.,* 2021) with a range of 3-6%. However, regardless of its geographical origin and variety, the short-stemmed Vasco variety exhibited a higher CP value (9.14%) compared to any untreated rice straw reported in the literature. Besides, only one study has examined the chemical composition of Vasco rice straw, and Akay (2022) reported a 7.03% CP content for the Vasco straw. Similar to our experiment, it was observed that CP values of short-stemmed varieties of Italian origins, such as Vasco, were higher than Cameo and Efe varieties. On the other hand, several studies comparing short and long varieties have reported that CP values are similar among varieties (Teimouri Yansari, 2017; Wahyono *et. al.*, 2021). There is significant genetic diversity among varieties depending on the quality of the straw. In addition to this genetic diversity, environmental conditions, cultivation methods, and other agronomic activities can directly affect the plant composition. Therefore, the inconsistent results observed in studies on rice straw may be attributed to an interaction between plant genetics and the environment (Van Soest, 2006; Ahmed *et al.,* 2022). The ash contents of Vasco, Cameo, and Efe straws were found 10.64%, 13.88%, and 14.97%, respectively. These values are lower than the reported ash values for California varieties (18.6%) (Abou-El-Enin *et al.,* 1999), Asian varieties (16.6%) (Nakashima and Ørskov, 1990), and Indonesian varieties (>20.0%) (Wahyono et. al., 2021). These findings are consistent with the study of Van Soest (2006), which reported that European rice varieties have lower silica content. Additionally, the lowest ash content was found in the Vasco variety. Silica is found in higher proportions in leaves compared to stem parts in rice straw (Agbagla-Dohnani, *et al.,* 2003). Short varieties are characterized by less leafiness compared to long varieties. Therefore, it is assumed that the lower ash content of Vasco compared to the other varieties is due to its lower leaf proportion.

In a study conducted by Agbagla-Dohnani *et al.* (2001), which compared 15 different European varieties of rice straw, NDF contents ranged from 76.3% to 81.4%, while ADF contents ranged from 44.5% to 53.1%. Although the ADF values of the varieties examined in our study were consistent with those reported by Agbagla-Dohnani *et al.* (2001), the NDF values were comparatively lower. Furthermore, the NDF and ADF contents of the Vasco, Cameo, and Efe varieties were lower than those reported by Akay (2022) for the same varieties. On the other hand, the NDF and ADF contents of the rice straw varieties in our study were found to be similar to those reported by Ravi *et al.* (2019) for 15 Indian-origin rice straw varieties.

The ADL contents found in Vasco (11.04 %), Cameo (10.83 %), and Efe (11.15 %) straws were comparable to those reported in 15 different European rice straw varieties investigated by Agbagla-Dohnani *et al.* (2001). Nevertheless, literature on rice straw varieties, predominantly from South Asia and the Far East, which constitutes a major part of the research, has reported lignin content in the dry matter to be below 8% (Jahromi *et al.,* 2010; Ravi *et al.,* 2019; Matias *et. al.,* 2019). These findings support Van Soest's (2006) assumption that European varieties have higher lignin content. The quality of rice straw is known to vary based on several factors, including the rice variety, cultivation method, and environmental conditions (Vadiveloo and Fadel, 2009). In recent years, rice breeding programs have focused on increasing grain yield rather than straw quality, resulting in the development of short varieties with lower straw yields. Despite concerns that shorter varieties would reduce straw availability for animal feed, research has indicated that short-stemmed varieties with high grain yields have higher digestibility, compensating for the lower straw yield (Van Soest, 2006). Compared to other cereal straws, rice straw has unique characteristics in terms of digestibility. Long-stemmed rice varieties tend to have more leaves, which are less digestible than the stem due to their higher silica content, while high-yielding short-stemmed varieties generally have more digestible straw (Van Soest, 2006; Dong *et al.,* 2013). In this study, the Vasco variety, which is the shortest and highest yielding, had the highest $IVTD_{DM}$ compared to other varieties, supporting previous research. However, there was no significant difference in $IVTD_{NDF}$ values among the varieties.

Nutrient composition and *in vitro* true digestibility of urea and/or crude glycerin-treated rice straw varieties: The present study findings elicited that urea treatment increased the CP levels of all rice straw varieties. These findings were consistent with studies that examined the treatment of rice straw with urea (Wanapat *et al.,* 2009; Abo-Donia *et al.,* 2021). Vadiveloo and Fadel (2009) reported that the treatment of 16 different Malaysian rice straw varieties with urea reduced the NDF level. Similarly, Jahromi *et al.* (2010) reported that the treatment of rice straw with urea reduced the NDF and hemicellulose contents. In line with these studies, we observed a decrease in the NDF and ADF contents of the Cameo and Efe varieties with urea treatment. However, this decrease did not occur in the Vasco variety. These findings support Vadiveloo's (2003) hypothesis that low-quality rice straw varieties respond better to urea treatment than high-quality straw varieties. The Vasco variety has a higher CP content and lower NDF and ADF contents, compared to other varieties, and can thus be considered of higher quality. The NDF and ADF are negatively correlated with feed digestibility (Syahniar *et al.,* 2018). According to a study conducted by Kour *et al.* (2023), the addition of urea at 1% and molasses at 6% resulted in a substantial drop ($p<0.05$) in NDF, hemicellulose, and cellulose, and a significant rise ($P<0.05$) in DM, ADL, acid detergent insoluble crude protein (ADICP), and neutral detergent insoluble crude protein (NDICP). Concerning the *in vitro* digestibility study, Kour *et al.* (2023) found that adding urea at 1%, molasses at 6%, Lactobacillus plantarum, and cocktail enzyme resulted in considerably higher ($p<0.05$) OMD%, DMD%, MCP%, and ME (MJ/kg DM).

Our findings indicate that urea treatment effectively increased $IVTD_{DM}$ and $IVTD_{NDF}$ in all rice straw varieties, except for the Vasco variety, which did not exhibit any changes in its NDF and ADF levels after treatment. Despite the observed decrease in NDF levels in the Cameo and Efe varieties, similar $IVTD_{NDF}$ values were obtained for the Vasco variety, possibly due to its lower NDF and ADF contents before treatment. Vadiveloo (2003) and Vadiveloo and Fadel (2009) reported an increase in $IVTD_{DM}$ of rice straw from 45% to 55-62% with 4% urea treatment. Similarly, Kumar *et al.* (2021) and Wanapat *et al.* (2009) reported that the $IVTD_{DM}$ of rice straw increased from 47-52% to 60-65% with 4% and 5.5% urea treatment, respectively. In line with these findings, our study showed that 5% urea treatment increased the $IVTD_{DM}$ and $IVTD_{NDF}$ of all varieties.

Molasses is commonly used as a readily soluble carbohydrate source to enhance the taste of rice straw and promote appropriate fermentation by stimulating microbial activity of lactic acid bacteria in the ensiling process (Cherdthong *et al.,* 2021; Kumar *et al.,* 2021). However, there is a lack of research regarding the utilization of crude glycerin as a potential sugar source during fermentation in the treatment of rice straw. Recently, crude glycerin has been safely used as an economical feed ingredient in ruminant diets, making it a promising alternative to molasses for rice straw treatment. Some studies have shown that the use of crude glycerin as a silage additive has improved the fermentative profile and nutritional value of various grass silages (Orricco *et. al.,* 2017; Schwingel *et. al.,* 2020). Hong *et al.* (2010) indicated that some anaerobic bacterial species can utilize glycerol as an energy source to produce organic acids, including lactic acid. Cunha *et al.* (2020) reported that treating Tifton 85 haylages with crude glycerin was more effective than some microbial inoculants (*L. plantarum, B.subtilis)* in improving fermentation parameters and nutritional value, even when the forage has high levels of dry matter. In a study conducted by Syahniar *et al.*(2018) wherein crude glycerin was used as a silage additive at a rate of 3% DM/kg to cassava leaves, the researchers reported that glycerol improved the quality of silage and did not have an adverse effect on *in vitro* rumen fermentation parameters. In our study, all treatment groups of the rice straw varieties, in which crude glycerin was used either alone or in combination with urea, showed a decrease in NDF and ADF levels. In contrast, Dias *et al.* (2014) reported that while both urea and crude glycerin significantly increased the $IVTD_{DM}$ value in sugarcane silage, only urea reduced the NDF content. Cell wall components are the primary limiting factors for digestibility in feed materials. During fermentation, fiber components can be hydrolyzed by acid, and the resulting carbon chain can be utilized as an energy source for the growth of lactic acid bacteria (Jatkauskas and Vrotniakienė, 2006). The decrease in NDF and ADF levels observed in straw treated with crude glycerin may be attributed to its ability to increase microbial activity during fermentation. This increase in microbial activity promotes the hydrolysis of cell wall components, leading to the observed decrease in NDF and ADF levels. However, despite the decrease in NDF and ADF levels, there was no significant improvement in the digestibility of all types of straws treated with crude glycerin. This lack of improvement in digestibility may be due to the adverse effects of external impurities present in crude glycerin on *in vitro* rumen fermentation.

Interaction effect of rice straw varieties and chemical treatments (urea and or crude glycerin) on the *in vitro* true digestibility ($IVTD_{DM}$) and *in vitro* NDF ($IVTD_{NDF}$) digestibility: The current study demonstrated that the interaction effect of Efe and urea+crude glycerin significantly increased (P<0.001) the *in vitro* true digestibility ($IVTD_{DM}$) compared to other interaction effects, as shown in Table 5. Lunsin *et al.* (2018) found that treating sugarcane bagasse with a combination of 5% urea and 5% molasses improved dry matter (DM) and organic matter (OM) digestibility, which led to enhanced *in vitro* true digestibility. The probable reason for this positive result can be due to the addition of nitrogen (urea) and energy (molasses) sources which increase the availability of soluble carbohydrates and nitrogen in silage (Bautista-Trujillo *et al.,* 2009; Ventura-Canseco *et al.,* 2012), resulting in the provoked activity of proteolytic microorganisms and improving digestibility (Zhang *et al.,* 2020). Similarly, this study presented that the interaction effect of Efe and urea significantly increased (P<0.001) *in vitro* true NDF digestibility ($IVTD_{NDF}$) compared to other interaction effects. Abo-Donia *et al.* (2022) also documented that treating rice straw with urea, molasses, and whey improved *in vitro* NDF, *in vitro* crude protein (CP), and *in vitro* digestibility of organic matter (OM) compared to untreated straw. This improvement in *in vitro* NDF and organic matter is attributed to the breakdown of carbohydrates in the presence of adequate nitrogen, which promotes increased microbial protein production (Bach *et al.,* 2005), which enhances the degradability of OM, NDF, CP, and microbial protein (MP) in ensiled rice straw. Increased digestibility of dry matter and OM may result from higher digestibility of acid detergent fiber and neutral detergent fiber (Anil *et al.,* 2000) and modifications in the cell wall and protein components of ensiled rice straw (Abo-Donia *et al.,* 2007; Hafez *et al.,* 2015). Elsheikh *et al.* (2020) noted that adding molasses to silage increased NDF digestibility due to enhanced cell wall hydrolysis. Abo-Donia *et al.* (2014) indicated that digestibility coefficients for most of the nutrients could be improved when roughages are treated biologically, thereby improving their feeding values, such as digestible crude proteins and total digestible nutrients, compared to untreated feed materials. The combination of effective treatment techniques and nutrient supplementation can improve the utilization of rice straw in a better way (Trach, 1998). Ventura-Canseco *et al.* (2012) explained that fermentation with added molasses and urea or whey breaks down some cell walls, reducing the proportions of NDF and ADF, and thus improving NDF digestibility.

Conclusion: The current study indicated that among the untreated rice straw varieties, the short-stemmed Vasco rice straw variety exhibited superior nutritional value compared to the Cameo and Efe varieties, owing to its high protein content and low NDF and ADF levels. Upon treatment with urea and crude glycerin, all varieties showed similar responses. However, significant increases in $IVTD_{DM}$ and $IVTD_{NDF}$ levels were observed in groups treated with urea alone and in combination with crude glycerin, while sole treatment with crude glycerin did not significantly affect digestibility. Utilizing crude glycerin as an alternative energy source for rice straw treatment has the potential to address the shortage of easily soluble energy sources in the treatment process.

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