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Effect of Urea and Exogenous Fibrolytic Enzyme Treated Paddy Straw on *In Vitro* Rumen Fermentation Characteristics and Degradability

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ABSTRACT

This study was conducted to evaluate the effect of urea and exogenous fibrolytic enzyme (EFE) treatment on *in-vitro* digestibility and fermentation attributes of paddy straw. In this study, *in vitro* degradability of nutrients in paddy straw as an effect of treatment of urea and EFE at various levels (1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0 and 9.0 % of urea and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 g/kg DM of EFE) were tested. For selecting the best levels of urea and EFE, test samples were incubated with strained rumen liquor and different dosages levels were studied on rumen fermentation parameters like total gas production along with *in vitro* DM and OM digestibility, ammonia nitrogen, pH and some calculated parameters such as partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial protein synthesis (EMPS) by incorporation of urea and EFE treated paddy straw. The result showed significant improvement on *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), *in vitro* total gas production (IVTGP), MBP and PF at 4% and 8 g/kg DM of urea and EFE treatment level in comparison to other levels. The optimum results were obtained at 4% and 8 g/kg DM of urea and EFE treated paddy straw, hence a 4% and 8 g/kg DM dosage, respectively may be selected for *in vivo* study.

KEYWORDS: Exogenous fibrolytic enzyme, *in vitro*, paddy straw, Rumen fermentation, urea

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INTRODUCTION

In many agricultural countries, paddy straw and other agro industrial by products are available in large quantities immediately after every harvest seasons. Thousand tonnes of agricultural residue is set on fire after the harvesting of the crop for preparation of field for the following crop. This problem is much severe in regions where farmers are practicing the mechanized rice-wheat cropping pattern (Mehta et al., 2013). Considerable effort has been made to improve the utilization efficiency and feeding value of cereal straws using pre-treatments to enhance its digestibility, including biological, chemical, and physical pre-treatments (Eun et al., 2006), as well as providing nutritional supplements based on the lack of nutrients in cereal straw (Wei et al., 2019). Ammoniation of rice straw by urea treatment may be suitable way for smallholder-farmers who keep animals in limited quantities because urea treatment can improve intake, crude protein and digestibility with low cost, relatively safe and easy to apply. Therefore, the use of rice straw as an animal feed as well as its treatment is always an economic decision (Sarnklong et al., 2010). Supplementing ruminant diets with fibre degrading enzymes has been shown to improve feed utilization and animal performance (Beauchemin et al., 2003). However, the effectiveness of feed enzymes in ruminant diets is dependent upon substrate-enzyme specificity. Thus, it is important to establish the optimum enzyme activities for the degradation of rice straw.

The aim of the present study was to evaluate the effect of urea and exogenous fibrolytic enzyme (EFE) treatment on *in vitro* fermentation attributes of paddy straw and to select the best levels of urea and EFE for the treating paddy straw for feeding ruminants.

MATERIAL AND METHODS

Urea and EFE were used in the trial i.e. neem coated urea and mafzyme forte. Mafzyme forte was purchased from Meenakshi Agro Farms, Bengaluru - 560043. Karnataka, India. Urea and

EFE were tested at 11 doses (1, 2, 3, 3.5, 4, 4.5, 5, 6, 7, 8 and 9%) and 9 doses (1, 2, 3, 4, 5, 6, 7, 8 and 9 g/kg DM), respectively to determine their effects on *in vitro* rumen fermentation. For *in vitro* trial, feeds were prepared from paddy straw milled by using 1mm screen.

Rumen liquor was collected from the cattle herd maintained at Livestock Farm Complex (LFC), DUVASU, Mathura. Animals in LFC were kept on similar feeding like straw and concentrate for about 3-5 days. The rumen liquor strained through four layers of muslin cloth in a glass flask. Flask kept in incubator prior to use and then the required amount of strained rumen liquor used as inoculums. Carbon dioxide gas was passed through the rumen liquor and maintained at 39±1ºC temperature for use of preparation of inoculum. 200 mg substrate was weighed in calibrated glass syringes (Super scientific Co. Bangalore) of 100 ml capacity. Sample was put on the bottom with the help of weighing boat with removable stem. For testing of each treatment group two syringes of substrate blank and two syringes as standard were prepared. The fermentation value for standard was checked with data base available in the laboratory. The incubation medium was prepared as described by Menke and Steingass (1988). 30 ml incubation medium (rumen fluid-medium mixture) was dispensed anaerobically in each preheated (39ºC) syringe. Syringes were closed using clamps and the volume of the mixture was recorded in the syringes. Then syringes were placed vertically in a wooden stand with hole to hold the syringes upright in the incubator ventilated by fan assisted forced air circulation at 39±0.5°C for 24 hr. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe from total gas produced in the syringe containing substrate and inoculum and buffer.

True DM degradability and OM degradability of feed sample of each syringe containing residues after incubation was estimated as per Van Soest et al. (1991). The partition factor (PF) is calculated as the ratio of substrate truly degraded *in vitro* (mg) to the volume of gas (ml) produced by it. It provides important information about partitioning of fermentation products. The microbial biomass production (MBP) was calculated by using the degradability of substrate and gas volume and stoichiometrical factor as suggested by Blummel et al. (1997). Daily microbial protein synthesis is the product of the efficiency of microbial protein synthesis (EMPS) (Hoover and Stokes, 1991), which usually is defined as grams of microbial crude protein (MCP) / kilogram or 100 grams of OM digested in the rumen (Hoover and Stokes, 1991). After 24 hr incubation, the pH of the rumen liquor was checked by pH meter. Ammonia nitrogen (NH_3-N) was estimated by using colorimetric method (Weatherburn, 1967).

$$MBP\ (mg) = TDOM\ (mg) - (gas\ volume \times 2.25)$$

$$EMPS\ (g/kg\ DOM) = MCP\ (g) / DOM\ (kg)$$

The generated data were statistically analyzed by ANOVA considering level of urea and EFE as factor using the general linear model procedure (univariate) using SPSS (SPSS for windows, Version 20.0; Inc., Chicago, IL, USA) software.

RESULTS AND DISCUSSION

The effect of urea treated paddy straw on *in vitro* rumen fermentation parameters was shown in Table 1. The *in vitro* dry matter digestibility (IVDMD %) and *in vitro* organic matter digestibility (IVOMD %) was significantly higher ($P<0.05$) in 4% urea treated paddy straw as compared to other dose levels of urea. The present results confirmed the results obtained by Ekani and Wahyono (2020) for urea treatments. Jayanegara et al. (2017) found that *in vitro* study of urea treated rice straw show increased in IVDMD and IVOMD by 18.0% and 17% as compared to control. Vadiveloo (2003) reported that treatment of low degradable variety of rice straw responded better to urea treatments than higher quality straw ultimately resulting increased *in vitro* DM degradability from 45 to 62%. Jayanegara et al. (2017) found increased values of IVOMD which might be due to high quickly soluble and fermentable protein leading to the rapid growth of microorganisms this helped in increasing the breakdown of OM. The *in vitro* total gas production (IVTGP, ml/200 mg DM) (IVTGP, ml/g DM) (IVTGP, ml/g digestible dry matter, DDM) were observed for various levels of urea treatment. The total gas production increases with increasing level of urea treatment. Abo-Donia et al. (2022) carried out *in vitro* study with urea treated rice straw and observed that there was

increase in gas production released. This increased gas release was associated with the improvement in the degradation of OM and NDF which resulted from the increased activity of microorganisms. Sniffen et al. (2006) reported that improved fiber degradation leads to an increase in gas accumulation as well as its release rate. During *in vitro* fermentation, the digestibility of feed OM is positively correlated with gas production. The higher the digestibility of OM, there was increase in the fermentation activity of microorganisms in the rumen, the gas production rate is accelerated, and the gas production is increased (Ma et al., 2020).

In the present study, the gas production of each urea treated groups were higher than that of the control group, indicating that the urea treatment can increase the soluble carbohydrate of rice straw and increase the gas production rate and the gas production. The results of *in vitro* studies further indicate that the higher the urea content, the greater gas production *in vitro*, which is consistent with the findings of Senthilkumar et al. (2010). In present study, pH did not differ significantly which was similar with the findings of Jihene et al. (2022) who also reported that the urea and Urea + EFE supplementation had no significant effects on ruminal pH. Sheikh et al. (2017) also reported non-significant difference in the rumen pH in sheep fed urea molasses treated rice straw. In the present study, ammonia nitrogen (mg/100 ml) and Total-N (mg/100 ml) were found significantly lower ($P<0.05$) at 4.0 % with compare to other levels when the TMR rations treated with urea. Total nitrogen and NH_3-N recorded at different hours post feeding the urea molasses treated rice straw were found significantly ($P<0.05$) higher in treatment groups than that of control (Sheikh et al., 2017). Gomaa et al. (2012) who revealed that lambs fed urea-molasses treated paddy straw has higher total nitrogen concentrations.

Table 1. Effect of urea treated paddy straw on *in vitro* rumen fermentation parameters

Parameters	Urea level (%)												Pooled SEM	P value
	Control	1.0	2.0	3.0	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0		
IVDMD (%)	71.46^{a}	71.90^{a}	73.30^{ab}	75.18^{b}	75.88^{b}	77.49^{c}	77.69^{c}	77.89^{c}	77.06^{c}	76.90^{bc}	75.19^{b}	75.25^{b}	2.56	0.038
IVOMD (%)	72.34^{a}	73.88^{a}	75.19^{ab}	76.70^{ab}	77.09^{ab}	79.12^{b}	78.89^{b}	77.15^{b}	78.31^{b}	77.28^{b}	76.61^{b}	77.17^{b}	1.95	<0.001
IVTGP (ml/200 mg DM)	29.52^{a}	30.53^{a}	31.97^{a}	34.29^{ab}	36.2^{b}	39.41^{c}	40.09^{c}	40.14^{c}	38.72^{c}	36.5^{b}	33.78^{ab}	29.42^{a}	1.16	0.011
IVTGP (ml/g DM)	189.25^{a}	195.7^{a}	206.25^{ab}	210.8^{b}	226.05^{c}	227.55^{c}	230.75^{c}	231.41^{c}	226.05^{c}	227.5^{c}	223.55^{bc}	189.25^{a}	14.29	0.045
IVTGP (ml/g DDM)	264.83^{b}	272.18^{bc}	281.38^{bc}	280.39^{bc}	297.90^{c}	293.65^{c}	297.01^{c}	297.10^{c}	293.34^{c}	295.84^{c}	297.31^{c}	251.50^{a}	13.66	0.004
pH	6.97	6.92	6.76	7.01	6.82	6.88	6.91	7.01	7.16	7.28	7.26	7.35	0.53	0.893
NH_3-N (mg/100 mL)	12.75^{ab}	12.50^{ab}	12.21^{a}	11.90^{a}	12.30^{ab}	11.89^{a}	12.31^{ab}	12.45^{ab}	13.05^{b}	14.55^{bc}	16.51^{c}	17.30^{d}	0.94	0.024
Total N (mg/100 ml)	52.25^{a}	54.00^{a}	56.24^{ab}	58.51^{ab}	60.22^{b}	63.18^{bc}	63.25^{bc}	65.51^{c}	67.26^{d}	67.39^{d}	68.25^{de}	70.51^{e}	2.52	0.006
PF	3.88	3.90	3.84	3.72	3.66	3.50	3.47	3.34	3.38	3.42	3.37	3.45	0.07	1.000
MBP (mg/200mg)	78.26^{e}	79.07^{e}	78.45^{e}	76.25^{e}	72.73^{bc}	69.57^{b}	67.58^{b}	63.99^{a}	69.50^{b}	72.44^{bc}	77.22^{e}	88.15^{d}	4.21	0.037
EMPS (g/kg DOM)	540.92^{fg}	535.11^{f}	521.66^{e}	497.05^{d}	471.72^{c}	439.63^{b}	428.30^{ab}	414.68^{a}	443.75^{b}	468.65^{c}	503.95^{d}	571.11^{g}	27.39	0.009

In present study NH_3 concentration was lowest for diets based on 4% urea-treated straw which was similar with findings of Trach et al. (2001) who reported rumen NH_3 concentration was lowest for diets based on 4% urea-treated straw. Akinfemi et al. (2020) estimated that gas volume at 24 hours of incubation progressively increased with increase in urea-molasses treatment which might be due to high crude protein in feed enhances microbial multiplication in the rumen, which in turn determines the extent of fermentation. Wanapat et al. (2013) reported that EMPS was enhanced by urea and urea-calcium hydroxide treated rice straw. In contrast, Sommart et al. (2000) found that MBP and EMPS were significantly lower in urea treated straw than cassava in an *in vitro* study using cassava, rice straw, urea treated rice straw and dried ruzi grass as substrates. MBP was found to be lowest in fermented rice straw (Yulia and Sari, 2021).

The effect of EFE mixture supplemented paddy straw on *in vitro* rumen fermentation parameters was shown in Table 2. Tang et al. (2008) found that fibrolytic enzyme supplementations improved the IVDMD and IVOMD of rice straw. Treatments of rice straw with increasing level of cellulase significantly improved IVDMD and IVOMD compared to untreated rice straw (Selcuk et al., 2016). Another reason might be due to the synergy between EFE and the ruminal flora and increase in ruminal bacteria (Kholif et al., 2022). Kumar et al. (2013) reported that fibrolytic enzymes had increasing effects on total gas production of feeds. Application of EFE both during feed ensiling and directly during feeding increased *in vitro* gas production (Selcuk et al., 2016). In the present study, increase in the amount of gas production might be due to the increase in the activity of rumen microorganisms resulting as an increase in OMD (Selcuk et al., 2016). Nitipot and Sommart (2003) stated that there was a positive correlation between the volume of gas released during fermentation and *in vitro* OMD. Sujani et al. (2017) reported that cellulase and xylanase enzymes were supplemented alone and as a mixture with rice straw stated that all enzymatic treatments enhanced the IVTGP significantly ($P<0.05$) when compared with the control, indicating active microbial fermentation. The IVTGP increased with increasing doses of enzymes (Sujani et al., 2017). Increased IVTGP was noted by Yang et al. (2011) for rice straw treated with fibrolytic enzymes, and Jalilvand et al. (2008) found similar outcomes for wheat straw supplemented with fibrolytic enzymes. Liu and Orskov (2000) found increased IVTGP in steam-treated rice straw supplemented with non-starch polysaccharide enzyme, which is consistent with the current findings.

In the present study NH_3-N concentration was decreases with supplementation of EFE. These results are in agreement with those reported by Silva et al. (2016) showing that the addition of xylanase to the dairy cow diet decreased the NH_3-N concentration. Likely, an *in vitro* trial conducted by Almaraz et al. (2016) stated that EFE supplementation of a diet decreased the ruminal NH_3-N by 11%. Jihene et al. (2022) showing the ruminal NH_3-N concentration decreased ($P<0.05$) by EFE supplementation alone. In the present study average concentration of NH_3 obtained were in the range from 11.49 to 13.17 (mg/100ml), still within the normal range of rumen microbial growth which was similar to earlier findings reported by Lamid et al. (2013) who stated that the average concentration of NH_3 obtained were in the range of 11.70 to 16.79 mg N/100ml.

Rumen pH, NH_3-N and VFA contents are vital parameters in ruminants that illustrate the normal functioning and steady state of the rumen (Jia et al., 2018), and VFA are principal products of rumen fermentation, which directly related to the balance of energy in ruminants (Sun et al., 2013). The addition of lignocellulolytic enzymes and bacterial lignocellulolytic on rice straw resulted in significant improvement of all the fermentation products (acetate, propionate, butyrate, and ammonia) between control and supplemented groups (Lamid et al., 2013).

Table 2. Effect of EFE mixture treated paddy straw on *in vitro* rumen fermentation parameters

Parameters	EFE mixture level (g/kg DM)										Pooled SEM	P value
	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0		
IVDMD (%)	51.03^{a}	51.52^{a}	51.88^{a}	52.62^{a}	54.99^{ab}	55.92^{ab}	58.42^{b}	60.02^{bc}	61.41^{c}	60.96^{bc}	6.39	0.017
IVOMD (%)	54.14^{a}	54.32^{a}	55.98^{a}	58.61^{ab}	59.99^{ab}	61.11^{b}	63.29^{c}	65.32^{d}	66.72^{d}	65.13^{d}	4.06	0.038
IVTGP (ml/200 mg DM)	30.18^{a}	30.51^{a}	31.99^{ab}	33.21^{b}	34.41^{b}	36.15^{c}	38.77^{d}	39.27^{d}	41.34^{e}	41.43^{e}	3.19	0.005
IVTGP (ml/g DM)	189.25^{c}	150.91^{a}	152.55^{a}	159.95^{ab}	171.9^{b}	176.5^{bc}	180.75^{c}	193.85^{cd}	196.35^{d}	206.7^{e}	12.53	<0.001
IVTGP (ml/g DDM)	370.86^{d}	292.90^{a}	294.04^{a}	303.97^{ab}	312.60^{b}	315.63^{b}	309.40^{b}	322.98^{bc}	319.74^{bc}	339.07^{c}	18.29	0.049
pH	6.19^{a}	6.22^{a}	6.34^{ab}	6.52^{ab}	6.69^{b}	6.79^{bc}	6.89^{c}	6.90^{c}	6.92^{c}	6.98^{c}	0.83	0.011
NH_3-N (mg/100 mL)	13.16^{b}	12.64^{ab}	13.23^{b}	12.99^{ab}	12.81^{ab}	12.44^{ab}	13.17^{b}	11.49^{a}	12.54^{ab}	12.39^{ab}	1.07	0.028
Total-N (mg/100 ml)	95.21^{a}	95.06^{a}	99.28^{b}	114.03^{c}	119.10^{c}	119.52^{c}	117.26^{c}	124.37^{cd}	129.39^{d}	130.51^{d}	6.29	0.044
TVFA (mM/100 ml SRL)	78.18^{a}	79.82^{a}	86.28^{ab}	97.23^{b}	99.12^{b}	112.25^{c}	116.89^{cd}	119.30^{cd}	126.16^{d}	125.85^{d}	7.41	0.039
Acetate (mM/100 ml)	50.82^{a}	51.88^{a}	56.08^{b}	63.20^{c}	64.43^{c}	72.96^{d}	76.22^{e}	77.55^{e}	82.00^{f}	81.80^{f}	4.50	0.008
Propionate (mM/100 ml)	19.55^{ab}	17.56^{a}	19.84^{ab}	23.34^{b}	22.30^{b}	27.39^{bc}	29.55^{c}	28.75^{c}	30.53^{c}	31.71^{c}	1.82	<0.001
Acetate: propionate	2.60:1	2.95:1	2.83:1	2.71:1	2.89:1	2.66:1	2.58:1	2.70:1	2.69:1	2.58:1	0.39	0.005
Butyrate (mM/100 ml)	7.91^{a}	7.82^{a}	10.38^{b}	10.35^{b}	10.56^{b}	10.60^{b}	11.90^{c}	11.49^{c}	13.00^{d}	13.63^{d}	1.48	0.033
PF	3.59	3.56	3.50	3.53	3.49	3.38	3.26	3.33	3.23	3.14	0.55	0.889
MBP (mg/200mg)	70.38^{a}	79.99^{b}	79.98^{b}	82.50^{bc}	92.56^{c}	90.88^{c}	79.35^{b}	82.28^{bc}	80.43^{b}	77.04^{ab}	4.18	0.018
EMPS (g/kg DOM)	649.98^{c}	736.28^{f}	714.36^{ef}	703.80^{d}	771.46^{g}	743.58^{g}	626.88^{b}	629.82^{b}	602.74^{ab}	591.43^{a}	36.29	0.047

$^{a-g}$Means within each column under the same subheading bearing different superscript letter are significantly different at P<0.05

Mohamed et al. (2005) evaluated the effect of an enzymatic mixture (cellulase, xylanase and protease) activities on the fermentation of substrate found that all acetate and propionate production were increased by all enzymatic treatments. Similarly, Giraldo et al. (2008) also stated that EFE supplementation increased propionate concentration.

Colombatto et al. (2007) studied the impact of fibrolytic enzymes on the rate and extent of fermentation of alfalfa stems (*in vitro*) and found that addition of these enzymes linearly increased *in vitro* OMD and DMD. Gado et al. (2007) evaluated the effect of biological treatments (cellulase; rumen liquor and Cellumonas cellulasae) of bagasse on lambs indicated that total VFA's values for treated bagasse by cellulase enzyme, rumen liquor and Cellumonas cellulasae were higher than that for untreated bagasse. Yang et al. (2000) revealed that the final concentration of VFAs increased by almost 9% with the enzymes. Arriola et al. (2011) found that supplementation of EFE @ 3.4 mg/g TMR dry matter to HF cows significantly increased TVFA concentration. EFE supplementation related to an increase in *in vitro* DMD and increases in the proportions of propionic acid and VFA (Gado et al., 2011). Kholif et al. (2022) reported an increased concentration of ruminal acetate with fibrolytic enzymes, which could be the result of improved apparent fiber degradation. Giraldo et al. (2007) reported that EFE supplementation significantly increased the microbial protein synthesis (MPS). Similar result obtained by Elwakeel et al. (2007) showing exogenous enzymes increase MPS which was an indicator that the bacterial population of the rumen is increased. Giraldo et al. (2008b) stated that supplementation of EFE increases the MPS which is justified that the EFE can release reducing sugars randomly which act as a readymade source of available energy and promotes rapid multiplication of the microbes (McAllister et al., 2001). Patel et al. (2015) reported that EFE supplementation was significantly increase total N concentration.

Table 3. Effect of urea and EFE mixture treated paddy straw on *in vitro* rumen fermentation parameters

Parameter	Treatment		Pooled SEM	P value
	0.0	Urea (4.0%) + EFE mixture (8.0 g/kg DM)		
IVDMD (%)	52.68[a]	62.93[b]	4.38	0.048
IVOMD (%)	53.93[a]	68.59[b]	3.92	0.002
IVTGP (ml/200 mg DM)	30.11[a]	43.18[b]	2.80	0.016
IVTGP (ml/g DM)	150.55[a]	215.90[b]	11.47	<0.001
IVTGP (ml/g DDM)	285.78[a]	343.08[b]	14.28	0.031
pH	6.47	6.58	0.42	0.938
NH_3-N (mg/100 mL)	11.69[a]	13.32[b]	1.06	0.014
Total-N (mg/100 ml)	93.21[a]	128.53[b]	8.39	0.028
PF	3.50[b]	2.91[a]	0.15	0.044
MBP (mg/200mg)	70.38[a]	79.99[b]	2.81	0.039
EMPS (g/kg DOM)	668.00[b]	635.55[a]	42.06	0.049

[a-b]Means within each column under the same subheading bearing different superscript letter are significantly different at P<0.05

Effect of urea and EFE mixture treated paddy straw on *in vitro* rumen fermentation parameters was shown in Table 3. In the present study, IVDMD, IVOMD and IVTGP were significantly increased. Similar observations found by Eun et al. (2006) and he stated that *in vitro* degradability of DM was greatly increased by addition of various types of enzymes along with ammonia treated straw indicating a synergistic effect between the ammonia treatment and enzyme application. The gas production was higher ($P<0.05$) in urea (4.0%) + EFE mixture (8.0 g/kg DM) treated paddy straw than that of the untreated paddy straw. The present study findings show non-significant difference in pH which was similar with the findings of Jihene et al. (2022) who also reported that the urea supplementation had no significant effects on ruminal pH. Sheikh et al. (2017) also reported non-significant difference in the rumen pH in sheep fed urea molasses treated rice straw along with fibrolytic enzymes. Author also reported that addition of enzymes in ammonia treated straw significantly improve IVTGP starting at 18 h of incubation, resulting in a 15-18% increase after 24 h. Wang et al. (2004) stated that alkali treatment alone significantly increased the amount of phenolic compounds, but not soluble carbohydrates, released from straw particles. In contrary, exogenous enzymes increased the release of soluble carbohydrates but not the release of phenolic compounds from straw particles.

Urea treatment/ ammoniation has been shown to give higher water- holding capacity to straw (Goto and Yokoe, 1996), resulting in softening of rice straw which was easily accessible to exogenous and rumen microbial enzymes. Thus, changes in the structural integrity of the cuticle by ammoniation/ urea treatment could facilitate the action of the exogenous enzymes, thereby resulting in substantial increases in rice straw degradation. Hence, ammonia or urea treatment of rice straw prior to enzyme application exerts the positive effects of enzymes, resulting enhanced microbial degradation of the rice straw (Eun et al., 2006). Jabri et al. (2022) reported improved IVTGP and IVOMD by addition of EFE to urea treated oat straw. This improvement is relevant for both the extent and the rate of gas production for so many studied enzymatic complexes, increasing estimated mostly used digestive parameters like OMD and VFA. Those findings were similar to the findings of kholif et al. (2022), and Abid et al. (2022) in which the EFE supplementation (xylanase and cellulase) effect on by-products commonly used for ruminant feeding (rice straw, barely straw, date palm leaves, and brewer's spent grain) were observed *in vitro* on OMD and microbial protein production.

CONCLUSION

The result showed improvement in *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, total gas production, microbial biomass production and partition factor. The optimum results were obtained at 4% and 8 g/kg DM of urea and EFE treated paddy straw, hence a 4% and 8 g/kg DM dosage, respectively may be selected for *in vivo* study.

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REFERENCES

Abid, K., Jabri, J., Yaich, H., Malek, A., Rekhis, J. and Kamoun, M. 2022. Study of the influence of exogenous fibrolytic enzyme additive on chemical composition, fermentation characteristics, and nutritional value of brewer's spent grain. Food Science and Nutrition. 10(6):1707.

Abo-Donia, F.M., Ahmed El-Shora, M., Abd-Elaziz Riad, W., Basuony Elgamal, N. and Abdel-Menaem El-Hamady, W. 2022. Improve the nutritional value and utilization of rice straw via an ensiling process with different sources of energy and nitrogen enrichment. Journal of Applied Animal Research. 50(1):333-41.

Akinfemi, A., Adebayo, B.J. and Ogunbosoye, D.O. 2020. Nutritional improvement of rice straw treated with urea-molasses and its effect on *in vitro* digestibility. Journal of Agricultural Science and Practice. 74-79.

Almaraz, I., González, S.S., Pinos-Rodríguez, J.M. and Miranda, L.A. 2016. Effects of exogenous fibrolytic enzymes on in sacco and *in vitro* degradation of diets and on growth performance of lambs. Italian Journal of Animal Science. 9(1):2-3.

Arriola, K.G., Kim, S.C., Staples, C.R. and Adesogan, A.T. 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. Journal of Dairy Science. 94 (2):832-841.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P. and Yang, W.Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. Journal of Animal Science. 81 (E. Suppl. 2):E37–E47.

Blummel, M., Makkar, H.P. and Becker, K. 1997. *In vitro* gas production: a technique revisited. Journal of Animal Physiology and Animal Nutrition. 77:24-34.

Colombatto, D., Mould, F.L., Bhat, M.K. and Owen, E. 2007. Influence of exogenous fibrolytic enzyme level and incubation pH on the *in vitro* ruminal fermentation of alfalfa stems. Animal Feed Science and Technology. 137(1-2):150-62.

Ekani, N. and Wahyono, T. 2020. Effect of different level of urea addition for rice straw fermentation application: *In vitro* evaluation. In IOP Conference Series: Earth and Environmental Science. 465(1):12016.

Elwakeel, E.A., Titgemeyer, E.C., Johnson, B.J., Armendariz, C.K. and Shirley, J.E. 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. Journal of Dairy Science. 90(11):5226-36.

Eun, J.S., Beauchemin, K.A., Hong, S.H. and Bauer, M.W. 2006. Exogenous enzymes added to untreated or ammoniated rice straw: Effects on *in vitro* fermentation characteristics and degradability. Animal Feed Science and Technology. 131(1-2):87-102.

Gado, H.M., Metwally, H.M., El Basiony, A., Soliman, H.S. and Abdel Galil, E.R. 2007. Effect of biological treatments on sugarcane bagasse digestibility and performance of baladi goats. Egyptian Journal of Nutrition and Feeds. 10:535-51.

Gado, H.M., Salem, A.Z.M., Odongo, N.E. and Borhami, B.E. 2011. Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. Animal Nutrition and Feed Technology. 165:131-136.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J., Ramos, S., Carro, M.D. 2008. Influence of direct-fed fibrolytic enzymes on diet digestibility and ruminal activity in sheep fed a grass hay-based diet. Journal of Animal Science. 86(7):1617-1623.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J. and Carro, M.D. 2008b. Effects of exogenous fibrolytic enzymes on *in vitro* ruminal fermentation of substrates with different forage: concentrate ratios. Animal Feed Science and Technology. 141: 306-325.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J. and Carro, M.D. 2007. Influence of exogenous fibrolytic enzymes and fumarate on methane production, microbial growth and fermentation in Rusitec fermenters. British Journal of Nutrition. 98: 753-761.

Gomaa, R., Gado, H.M., El-Sayed, H. and Abd El Mawla, S. 2012. Usage of treated rice straw with exogenous anaerobic bacterial enzymes (ZAD) for Ossimi sheep. Annals of Agricultural Sciences. 57:183-190.

Goto. M. and Yokoe, Y. 1996. Ammoniation of barley straw: effect of cellulose crystallinity and water-holding capacity. Animal Feed Science and Technology. 58:239-247.

Hoover, W.H. and Stokes, S.R. 1991. Various factors affecting microbial protein synthesis in the rumen. Journal of Dairy Science. 74:3630-45.

Jabri, J., Ammar, H., Abid, K. et al. 2022. Effect of exogenous fibrolytic enzymes supplementation or functional feed additives on *in vitro* ruminal fermentation of chemically pre-treated sunflower heads. Agriculture.12(5):696.

Jalilvand, G., Odongo, N.E., López, S., Naserian, A., Valizadeh, R., Shahrodi, F.E., Kebreab, E. and France, J. 2008. Effects of different levels of an enzyme mixture on *in vitro* gas production parameters of contrasting

forages. Animal Feed Science and Technology. 146(3-4):289-301.

Jayanegara, A., Ayinda, R.S. and Laconi, E.B. 2017. Urea treatment of rice straw at elevated temperature and pressure: Effects on fiber content, rumen fermentation and digestibility. Journal of the Indonesian Tropical Animal Agriculture. 42(2):81-87.

Jia, P., Cui, K., Ma, T., Wan, F., Wang, W., Yang, D., Wang, Y., Guo, B., Zhao, L. and Diao, Q. 2018. Influence of dietary supplementation with *Bacillus licheniformis* and *Saccharomyces cerevisiae* as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs. Scientific Reports. 8(1):16712.

Jihene, J., Khalil, A., Samia, B.S., Hela, Y., Atef, M., Jamel, R. and Mohamed, K. 2022. Effect of fibrolytic enzyme supplementation of urea-treated wheat straw on nutrient intake, digestion, growth performance, and blood parameters of growing lambs. Small Ruminant Research. 217:106840.

Kholif, A.E., Gouda, G.A., Morsy, T.A., Matloup, O.H., Fahmy, M., Gomaa, A.S. and Patra, A.K. 2022. Dietary date palm leaves ensiled with fibrolytic enzymes decreased methane production, and improved feed degradability and fermentation kinetics in a ruminal *in vitro* system. Waste and Biomass Valorization. 13(8):3475-88.

Kumar, S., Dagar, S.S., Sirohi, S.K., Upadhyay, R.C. and Puniya, A.K. 2013. Microbial profiles, in vitro gas production and dry matter digestibility based on various ratios of roughage to concentrate. Annals of Microbiology. 63(2):541-45.

Lamid, M., Ni Nyoman, T.P. and Sarwoko, M. 2013. Addition of Lignocellulolytic Enzymes Into Rice Straw Improves In Vitro Rumen Fermentation Products. Journal of Appllied Environmental and Biological Sciences. 3(9):166-171.

Liu, J.X. and Ørskov, E.R. 2000. Cellulase treatment of untreated and steam pre-treated rice straw-effect on *in vitro* fermentation characteristics. Animal Feed Science and Technology. 88(3-4):189-200.

Ma, Y., Chen, X., Zahoor Khan, M., Xiao, J., Liu, S., Wang, J., He, Z., Li, C. and Cao, Z. 2020. The impact of ammoniation treatment on the chemical composition and *in vitro* digestibility of rice straw in Chinese Holsteins. Animals. 10(10):1854.

McAllister, T.A., Hristov, A.N., Beauchemin, K.A., Rode, L.M. and Cheng, K.J. 2001. Enzymes in ruminant diets. Enzymes in Farm Animal Nutrition. 273-98.

Mehta, C.R., Sharma, S., Nair, R. and Singh, K.P. 2013. Impact of crop residue burning on environment and human health. Indian Farming. 63(5):24-25.

Menke, K.H. and steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analyses and gas production using rumen fluid. Animal Resources Development. 28:7-55.

Mohamed, A.H., El-Said, B.E., Ibrahim, Y.K., Tejido, M.L. and Carro, M.D. 2005. Effect of exogenous enzymes on *in vitro* ruminal fermentation of a high forage diet and productive response of lactating ewes. Egyptian Journal of Nutrition and Feeds. 8:591-602.

Nitipot, P. and Sommart, K. 2003. Evaluation of ruminant nutritive value of cassava starch industry by products, energy feed sources and roughages using *in vitro* gas production technique. In Proceeding of Annual Agricultural Seminar. 27(28): 179-190.

Patel, B.C., Oza, R.S., Desai, V.R and Gupta, R.S. 2015. Effect of fibrolytic enzyme on nutrient utilization and rumen fermentation pattern in sheep. Journal of Animal Research. 5(4):807-11.

Sarnklong, C., Cone, J.W., Pellikaan, W. and Hendriks, W. H. 2010. Utilization of rice

straw and different treatments to improve its feed value for ruminants: a review. Asian-Australasian Journal of Animal Sciences. 23(5):680-692.

Selcuk, Z., Salman, M. and Cetinkaya, N. 2016. The Effect of Cellulase Enzyme Treatment on Digestibility of Rice Straw. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 22(1).

Senthilkumar, S., Valli, C. and Balakrishnan, V. 2010. *In vitro* ruminal gas production of enzyme treated versus ureatreated paddy straw. Indian Veterinary Journal. 87:584-586.

Sheikh, G.G., Ganai, A.M., Sheikh, F.A., Bhat, S.A., Masood, D., Mir, S., Ahmad, I. and Bhat, M.A. 2017. Effect of feeding urea molasses treated rice straw along with fibrolytic enzymes on the performance of corriedale Sheep. Journal of Entomology and Zoology Studies. 5(6):2626-2630.

Silva, T.D., Takiya, C.S., Vendramini, T.H., de Jesus, E.F., Zanferari, F. and Rennó, F.P. 2016. Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows. Animal Feed Science and Technology. 221:35-43.

Sniffen, C.J., Ballard, C.S., Carter, M.P., Cotanch, K.W., Dann, H.M., Grant, R.J., Mandebvu, P., Suekawa, M. and Martin, S.A. 2006. Effects of malic acid on microbial efficiency and metabolism in continuous culture of rumen contents and on performance of mid-lactation dairy cows. Animal Feed Science and Technology. 127(1-2):13-31.

Sommart, K., Parker, D.S., Rowlinson, P. and Wanapat, M. 2000. Fermentation characteristics and microbial protein synthesis in an *in vitro* system using cassava, rice straw and dried ruzi grass as substrates. Asian-Australasian Journal of Animal Sciences. 13(8):1084-93.

SPSS. 2011. Statistical Package for Social Science. Base system user's guide, version 20.0. SPSS, Armonk, NY, IBM Corp, USA.

Sujani, S., Piyasena, T., Seresinhe, T., Pathirana, I. and Gajaweera, C. 2017. Supplementation of rice straw (*Oryza sativa*) with exogenous fibrolytic enzymes improves *in vitro* rumen fermentation characteristics. Turkish Journal of Veterinary and Animal Sciences. 41(1):25-29.

Sun, P., Wang, J.Q. and Deng, L.F. 2013. Effects of *Bacillus subtilis* natto on milk production, rumen fermentation and ruminal microbiome of dairy cows. Animal. 7(2):216-22.

Tang, S.X., Tayo, G.O., Tan, Z.L., Sun, Z.H., Shen, L.X., Zhou, C.S. and Shen, S.B. 2008. Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. Journal of Animal Science. 86(5): 1164-1172.

Trach, N.X., Mo, M. and Dan, C.X. 2001. Effects of treatment of rice straw with lime and/or urea on its chemical composition, in-vitro gas production and *in-sacco* degradation characteristics. Livestock Research for Rural Development. 13:5-12.

Vadiveloo, J. 2003. The effect of agronomic improvement and urea treatment on the nutritional value of Malaysian rice straw varieties. Animal Feed Science and Technology. 108:33-146.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74(10):3583-97.

Wanapat, M., Kang, S., Hankla, N. and Phesatcha, K. 2013. Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows. African Journal of Agricultural Research. 8 (17):1677-1687.

Wang, Y., Spratling, B.M., Wiedmeier, D.R. and McAllister, T.A. 2004. Effect of alkali pretreatment of wheat straw on the efficacy of exogenous fibrolytic enzymes. Journal of Animal Sciences. 82:198-208.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical chemistry. 39(8):971-74.

Wei, Z.H., Liang, S.L., Wang, D.M., Liu, H.Y., Wanapat, M. and Liu, J.X. 2019. Lactation performance and rumen fermentation in dairy cows fed a diet with alfalfa hay replaced by corn stover and supplemented with molasses. Asian-Australasian Journal of Animal Sciences. 32(8):1122.

Yang, H.E., Son, Y.S. and Beauchemin, K.A. 2011. Effects of Exogenous Enzymes on Ruminal Fermentation and Degradability of Alfalfa Hay and Rice Straw. Asian-Australasian Journal of Animal Sciences. 24(1):56-64.

Yang, W.Z., Beauchemin, K.A. and Rode, L.M. 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. Journal of Dairy Science. 83(11):2512-20.

Yulia, E. and Sari, D.K. 2021. Effect of addition cattle feed supplement on *in vitro* fermentation, synthesis of microbial biomass, and methane production of rice straw fermentation basal diets. In IOP Conference Series: Earth and Environmental Science. 888(1):012070.