EFFECT OF TYPE OF TREATED RICE STRAW ON RUMINAL FERMENTATION CHARACTERISTIC AND CELLILOLYTIC BACTERIA OF SWAMP BUFFALO

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ABSTRACT

Rumen microorganism community population and diversity in the swamp buffaloes (*Bubalus bulalis*) were assessed by 4 types of treated rice straw. Four, yearling swamp buffalo were randomly assigned to a 4x4 Latin square design. The animals were fed with concentrate at 0.5%BW and were offered *ad libitum* with roughages, rice straw (RS), urea treated rice straw (UTS), yeast treated rice straw (YTS) and urea-lime treated rice straw (ULTS). It was found that intake and ruminal NH_3-N were significantly different among dietary treatments and were found higher in swamp buffalo fed with YTS ($P<0.05$). Total VFAs were significantly different with roughage in swamp buffalo. Molar proportions of individual VFA in swamp buffalo were not affected by roughage sources. Feeding with YTS or ULTS increased cellulolytic bacteria population, particularly, the distribution of predominant bacteria; *F. succinogenes*, *R. flavefaciens*, and *R. albus*, respectively. The results from this study suggest that swamp buffaloes fed on yeast treated rice straw could improve rumen fermentation efficiency, rumen fibrolytic microbes' quantity and rumen ecology for the host buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, swamp buffalo, rumen microorganism, treated rice straw, yeast, molecular technique

INTRODUCTION

Livestock production, particularly buffalo, is critical to the economic and social fabric of smallholder farmers and sustainable agricultural systems, especially among the landless rural poor. They are used as draft power, means of transportation, capital, credit, meat, milk, social value, hide, and source of organic fertilizer (Chantalakhana, 2001; Devendra, 2007; Nada and Nakao, 2003). Although it is recognized that feeds resources in tropical areas are scarce in both quantity and quality especially in the dry season, their contribution is incredibly relevant. This is particularly true in topics where buffaloes and cattle are raised as part of crop production, and rice is the most important available commodity, from which by-products are used as feed (Wanapat *et al*., 2000; Wanapat *et al*., 2017). In rice-producing Asian countries including Bangladesh, China, India, Indonesia, Vietnam, and Thailand, rice straw is a major agricultural residue. However, rice straw has limited and unbalanced nutritive content, high lignin and silica content, and low crude protein content, both of which contribute

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to low voluntary intake and rate of digestion (Van Soest, 2006). For several years, numerous extensive studies have attempted to improve the nutritional content of rice straw through physical, chemical, and microbial treatments. Various physical and chemical methods may be used to facilitate the use of rice straw as ruminant feed (Wanapat, 2009; Khejornsar and Wanapat, 2011). The quality improvement by biological treatment is a fascinating solution since it is less harmful to the atmosphere and safer for animals than chemical approaches. According to Wang *et al.* (2016), yeast inoculants have been shown to increase the effectiveness of cereal straw utilization and ruminant efficiency. Fibrous agricultural residues contain a large pool of structural carbohydrate that can be degraded into volatile fatty acids by rumen microbes. There are the most major disadvantages found when using these resources as feed. As a result, adequate treatments and/or supplements are needed to compensate for the shortfalls and increase feeding effectiveness (Khejornsart and Wanapat, 2011). It has previously been reported that swamp buffalo utilized feed more effectively than cattle when kept under the same conditions, and that the diversity of rumen microorganisms increased when feeding different roughage (Wanapat and Cherdthong, 2009; Khejornsart and Wanapat, 2010). As a consequence, new molecular methods with the accuracy and enumeration of rumen microorganisms have been commonly applied. However, there has been relatively little study on the impact of alkaline and microbial treatments of rice straw silage on fermentation characteristics and microbes in the rumen of swamp buffalo by using molecular techniques. Therefore, the aim of this study was to compare the feed intake and rumen microorganism composition using real-time PCR techniques, as well as the fermentation of rumen by various treated rice straw in swamp buffalo.

MATERIALS AND METHODS

Ensiling materials and silage preparation

The current study was conducted at the Department of Agriculture and Resources, Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand, from March 2020 to June 2020. The rice straw (*Oryza sativa* L.) was obtained and thrashed to remove the grain at the maturity stage using a combined harvester (DC-70, Kubota, Osaka, Japan). The rice straw treatments were as follows: Urea, Urea-lime and ureatreated rice straw were prepared by the method of Wanapat *et al.* (2009), using 2 kg of urea and 2 kg lime mixed with 100 kg of water and poured over a stack of 100 kg rice straw then covered with a plastic sheet for a minimum of 14 days before feeding to the ruminants. For yeast treated rice straw were used 5 L starter of the *Saccharomyces cerevisiae* with 1.0×10^8 CFU /mL was diluted with 3 kg g of molasses and 1 kg of urea in 100 L of solution, then added in a 100 kg of rice straw by the spraying over a stack then covered with a plastic sheet for a minimum of 14 days.

Experimental design, diets and management of animals

The study was conducted in Animal Science Research Farm (Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Thailand). The experimental protocol was performed in accordance with the practices outlined in the Guide for the Care and Use of Kasetsart University. Four yearling swamp buffaloes (*Bubalus bubalis*) (269±28 kg of BW) were used in a 4x4 Latin squares design over four 21 days period. They were housed in individually penned and had free access to water during the experiment. The four dietary treatments were (i) untreated rice straw (RS), (ii) 2% urea treated rice straw (UTS), (iii) yeast treated rice straw (YTS), (iv) 2x2% urea-lime treated rice straw ULTS. Concentrate was fed at a 0.5% body twice a day at 07:00 and 16:00 with fed *ad libitum* access to treated straw or untreated straw. The concentrates are comprised 14% CP, and 65% TDN with required levels of minerals (Table 1).

Sampling procedures and chemical analysis

Each experimental period, feed and refusal were collected for intake evaluation. Composited of dietary samples were analyzed for DM, EE, ash and CP content (AOAC, 2012), NDF and ADF (Van Soest *et al*., 1991). Rumen fluid and content were collected after feeding (0, and 4h) in the last day of each period. Rumen fluid was measured for pH (HI 8424 microcomputer pH meter, HANNA Instruments, USA) immediately, and for later analysis of NH_3-N concentration by Kjeltec Auto 1030 Analyzer (Foss Analytical A/S, Denmark). Ruminal volatile fatty acids (VFAs) concentration were measured using high pressure liquid chromatography (HPLC) (Agilent 1200 series, Agilent Technologies Inc. Santa Clara, CA, USA) with diode array detector, Zorbax Eclipse XDB-C18 column (4.6 x150 mm., 5 µm) using 0.1M phosphate buffer as mobile phase. Ruminal content was separated for DNA extraction by the RBB+C method (Yu and Morrison, 2004) and populations of total bacteria, *F. succinogenes, R. albus, and R. flavefaciens* were expressed as a proportion of total rumen bacterial were determined by real-time PCR with specific primer according to Koike and Kobayashi (2001). Primer for total bacteria, forward primers (5-CGGCAACGAGCGCAACCC-3'), reverse primers (5'-CCATTGTAGCACGTGTGTAGCC-3'); *F. succinogenes*, Fs219f(5'-GGTATGGGATGAGCTTGC-3') and Fs654r(5'-GCCTGCCCCTGAACTATC-3'); *R. albus* primers, Ra1281f(5'-CCCTAAAAGCAGTCTTAGTTCG-3') and Ra1439r(5'-CCTCCTTGCGGTTAGAACA-3') and for *R. flavefaciens* primers, Rf154f (5'-TCTGGAAACGGATGGTA-3') and Rf425r (5'-CCTTTAAGACAGGAGTTTACAA-3').

Statistical analysis

The data were analyzed in a 4×4 Latin square design by analysis of variance run in the PROC GLM of SAS (SAS, 1998). Data were analyzed using the model:

$$Yijk = \mu + Mi + Aj + Pk + \varepsilon ijk$$

Where Yijk, observation from animal j, receiving diet i, in period k; µ, the overall mean; Mi, effect of roughage source (i = RS, UTS, YTS, and ULTS); Aj, the effect of animal (j = 1, 2, 3 and 4); Pk, the effect of period (k = 1, 2, 3 and 4); εijk, the residual effect. The results were presented as mean values and standard error of the means. Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of feeds: The experimental feed ingredients and their chemical composition during the 4 experimental periods is shown in Table 1. The percentage composition of the treated and untreated rice straw given to buffaloes were similarly value for urea-lime treated rice straw has been found by Wanapat *et al*. (2009). On the other hand, Shen *et al*. (1999) have been reported degradation of cellulose and hemicellulose with decrease in organic and dry matter losses when straw was treated with 5% urea. As reported by Khejornsart and Wanapat (2010); Khejornsart and Wanapat (2011) the treatment with NaOH, urea and lime increased gas production and rate constant of GP were highest for NaOH, follow by 2% urea +2% lime, 3% urea, 3% lime, 3.5% urea +3.5% lime and lowest for 5% urea treated rice straw. Wanapat *et al*. (2009) pointed that chemical treatments increased digestibility of low-quality roughages and sodium hydroxide treatment had stronger effect on cell wall dissolve than urea and calcium hydroxide. It is essentially a delignification process that involves eliminating the linkages between carbohydrates and lignin in order to solubilize a large amount of hemicellulose. Moreover, it physically makes structural fibers swollen and thereby increases the number of accessible sites of microbial attachments on the surface of the particles to have higher degradability and better intake by animals. Urea treatment is a conventional and widely used pretreatment of rice straws that increases the nitrogen content of ensiling materials that are nonhazardous and acts as a delignifying agent by ammonification, raising protein content and digestibility (Khejornsart and Wanapat, 2010; Khejornsart and Wanapat, 2011; Man and Wiktorsson, 2001; Zhang *et al*., 2019).

Dry matter feed intake was significantly highest in yeast treated rice straw (P<0.05). There has been reported in previous work of the two species, although, slightly higher intakes in swamp buffaloes than in Brahman crossbred cattle were found when fed on rice straw, urea, or concentrate (Van Soest, 2006; Wanapat *et al*., 2009). Zhang *et al*. (2019) found that urea plus nitrate pretreated rice straw could intake and neutral detergent fiber digestibility of goat. Wanapat *et al*. (2013) show that the 2+2% urea-lime and 3% urea treated rice straw could improve nutritive value of rice straw through increasing the DM intake, nutrient digestibility, rumen ecology, and milk yield and composition in lactating dairy cows. It is also reported by Trach et al. (2001) that 5% UTS and 3.5% ULTS treatments could increase in rumen microbe population and nutrient digestibility in *in vivo* trials. However, Khejornsart and Wanapat (2011) reported that chemical improvement by urea, lime or sodium hydroxide not effected on intake of swamp buffalo. Increased dry matter intake can provide the microbial population with sufficiently soluble growth factors, such as organic acids, B vitamins, and amino acids, according to the findings of this research. Supplementation of yeast increases ruminant feed digestion in a variety of ways, including rising nutrient digestibility, optimizing ruminal volatile fatty acid (VFA) proportion, reducing ammonia nitrogen (NH_3-N), alleviating pH fluctuation, and stimulating the population of ruminal microorganisms, according to several researchers (Wang *et al*., 2016; Chaucheras-Durand *et al*., 2019).

Ruminal pH of swamp buffaloes in all treatments were significantly increased in urea-lime treated rice straw and follow by urea treated rice straw. All treatment means were within the normal range (6.3 to 7.0) as optimal use for microbial digestion of fiber. The rumen pH of buffaloes supplemented with each of the treatment feeds, as well as supplementation of concentrate, was found to be suitable for fiber fermentation and microbial protein synthesis in this research.

Ruminal ammonia nitrogen (NH_3-N) has been recognized as an essential nutrient in rumen fermentation. Both ruminal NH_3-N in YTS and ULTS group was higher than in the control group (P<0.05) and was similar to report of Wanapat and Pimpa (1999). In order to Wanapat *et al.* (2000) have shown that swamp buffaloes are more efficient than cattle in many aspects, namely N-recycling and fiber digestion, ruminal NH_3-N level in relation to efficient fermentation and intake. This is in agreement with Cherdthong *et al.* (2010), who stated that the synthesis of ruminal microbial bacteria depends on an adequate supply of carbohydrate as an energy source and NH_3-N for peptide bond synthesis. Total VFA concentrations and molar individual VFA were not significantly different with treated rice straw. Study by Wanapat *et al.* (2009) who reported that ruminal total VFA and molar individual VFA were not affected by energy sources and urea level. Nair *et al.* (2019) found the reducing of C2 when diary heifer fed on corn silage inoculated with *S. Cerevisiae*, whereas Bakr *et al.* (2015) found that increasing TVFA and C3 concentrations in dairy cows fed with *S. cerevisiae*. The addition of *S. cerevisiae* also changed the molar proportion of VFA specifically the C3 concentration in the rumen, leading to an increase in the potential glucogenic for ruminants.

The results of real-time PCR revealed that total bacteria, *F. succinogenes*, *R. flavefacience* and *R. albus* were higher in swamp buffalo fed YTS than in another group (P<0.05). Cellulolytic bacteria population were increased from 0 to 4 hours post feeding. Predominant bacteria as *F. succinogenes*, *R. flavefacience* and *R. albus* were ranged from $2.35x10^{12}$-$2.80x10^{12}$, $0.74x10^{10}$-1.30×10^{10}, $0.78x10^{10}$-$2.62x10^{10}$ copies/mL of rumen content, respectively. There were found the population of *F. succinogenes* was highest across dietary treatments as compared to *R. flavefaciens* and *R. albus*. These findings were consistent with studies by Koike and Kobayashi (2001); Khejornsart *et al.* (2001); Wanapat and Cherdthong (2009) found that *F. succinogenes* to be the most abundant of the cellulolytic species in the swamp buffalo rumen, followed by *R. flavefaciens* and consequently by *R. albus*. Obviously, sine *F. succinogenes* and *R. flavefaciens* can colonize the cellulose more rapidly than the other 2 species and it can grow more rapidly on cellodextrins (the intermediate products of cellulose hydrolysis). *R. albus* was often less abundant than *F. succinogenes* and *R. flavefaciens* because it was less efficient in cellulose colonization and was probably limited to growing on soluble products released during cellulose hydrolysis by other organisms (Weimer, 1998; Wanapat *et al.*, 2009). It may also be explained by yeast-treated rice straw providing sufficiently soluble growth factors for rumen microbes, resulting in increased fiber degradation, which can increase feed intake and average daily gain. Yeast has also been related to increased fiber digestion, increased microbial protein flow, and activation of cellulolytic and lactate-using bacteria in the rumen.

CONCLUSION

Based on this experiment, it could be concluded that when buffaloes were adopted under yeast treated rice straw, buffaloes utilized feed more efficiently with higher rumen fermentation efficiency, NH_3-N and cellulolytic bacteria population. Yeast treated rice straw for buffalo can be a sustainable and effective forage sources, particularly during dry season in Thailand. Further performance studies in term on meat and milk

Table 1. The gradient and chemical composition (%) of concentrate and various treated rice straw were used in the experiment.

Items	Concentrate	RS	UTS	YTS	ULTS
Ingredients					
Cassava chip	60.0	-	-	-	-
Rice bran	4.5	-	-	-	-
Brewer's grain	4.0	-	-	-	-
Palm kernel meal	12.0	-	-	-	-
Soy bean meal	8.0	-	-	-	-
Leucaena leaf meal	8.0				
Molasses	2.0	-	-	-	-
Urea	0.5	-	-	-	-
Mineral mixed*	0.5	-	-	-	-
Salt	0.5	-	-	-	-
Chemical composition, %DM					
DM	89.6	90.6	55.2	50.9	54.9
OM	91.1	87.2	89.8	88.9	87.4
CP	14.9	2.0	5.8	6.2	5.6
NDF	9.2	77.0	70.4	68.7	69.9
ADF	9.5	56.0	56.9	50.5	52.5

RS = rice straw, UTS = urea treated rice straw, YTS = yeast treated rice straw, ULTS = urea-lime treated rice straw, DM = dry matter, CP = crude protein, EE = ether extract, OM = organic matter, NDF = neutral detergent fiber, ADF = Acid detergent fiber.

*Minerals and vitamins (each kg contains): vitamin A, 10 000 000 IU; vitamin E, 70 000 IU; vitamin D, 1 600 000 IU; Fe, 50 g; Zn, 40 g; Mn, 40 g; Co, 0.1 g; Cu, 10 g; Se, 0.1 g; I, 0.5 g.

Table 2. Effect of rice straw treated by urea, yeast and urea-lime on intake and ruminal fermentation parameters of swamp buffalo.

Parameters	RS	UTS	YTS	ULTS	SEM	P-value
Feed intake						
kg DM /d	5.60^{b}	5.62^{b}	7.15^{a}	7.09^{a}	0.259	0.029
%BW	1.60	1.69	1.90	1.82	0.180	0.186
g/kg $BW^{0.75}$	64.41^{a}	64.54^{a}	80.18^{b}	79.95^{b}	6.601	0.012
Ruminal pH	6.66	6.76	6.68	6.87	0.155	0.045
Ruminal NH_3-N, mg%	9.54^{ab}	12.32^{ab}	18.51^{c}	16.09^{bc}	0.829	0.021
Total VFA, mM/L	112.45	112.42	114.58	113.12	6.194	0.415
VFA, mol/100mol						
Acetate, C2	69.78	68.61	69.05	68.76	2. 391	0.862
Propionate, C3	19.89	20.32	20.64	20.39	1.520	0.068
Butyrate, C4	10.33	11.07	10.31	10.85	0.977	0.106
C2:C3	3.51	3.38	3.35	3.37	0.251	0.541

RS = rice straw, UTS = urea treated rice straw, YTS = yeast treated rice straw,
ULTS = urea-lime treated rice straw.
$^{a-c}$values in the same row with different superscripts with differ ($P<0.05$).

Table 3. Effect of different type of rice straw treatment on quantification of rumen bacteria by using real-time PCR (Mean±SE).

DNA copies of microbes	RS	UTS	YTS	ULTS	SEM	P-value
Total bacteria, x 10^{12}/ml	3.72^{b}±0.52	4.09^{b}±0.72	5.39^{a}±0.15	5.23^{a}±0.13	0.098	0.040
F. succinogenes, x 10^{12}/ml	2.35±0.84	2.67±0.90	2.80±0.01	2.39±0.35	0.418	0.138
R. albus, x 10^{10}/ml	0.78^{b}±0.27	0.62^{b}±0.24	2.62^{a}±0.21	0.59^{b}±0.08	0.250	<0.001
R. flavefaciens, x 10^{10}/ml	1.16^{a}±0.74	1.30^{a}±0.47	0.89^{b}±0.09	0.74^{b}±0.18	0.142	<0.001

RS = rice straw, UTS = urea treated rice straw, YTS = yeast treated rice straw,
ULTS = urea-lime treated rice straw
$^{a-c}$values in the same row with different superscripts with differ ($P<0.05$)

quality, feeding rice straw with yeast fermentation could be conducted which a good option to meet the increasing demand for feed resources with high potential utilization and low environmental affect for buffalo production.

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