



## **Effect of Substitution of Urea with Different Types and Levels of Ruminant Manure on Microbial Evaluation of Rice Straw Silage**

**Ali A. Saeed <sup>1\*</sup> & Saja I. Abid <sup>2</sup>**

<sup>1</sup>Department of Animal Production, College of Agriculture, University of Al-Qasim Green, Iraq

<sup>2</sup>Department of Animal Production, College of Agriculture, University of Wasit, Iraq

\*Corresponding author e-mail: [draliameensaheed59@agre.uoqasim.edu.iq](mailto:draliameensaheed59@agre.uoqasim.edu.iq)

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**Abstract:** This study was conducted in Nutrition Lab. Department of Animal Production, College of Agriculture, Al-Qasim Green University to investigate the effect of type and level of substitution of urea with ruminant manure (sheep, cow and buffalo) on basis of nitrogen content on microbial composition of rice straw silage. Urea was substituted with dried manure at six combinations, 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50. Silage samples were prepared by treating chopped straw with pre-treated solution contained 10% low quality juice and 2% urea. Treated straw was packed in double plastic bags which were closed tightly and fermented for 60 days in bits. Results revealed that samples prepared by addition of cow manure were characterized with higher number of total anaerobic ( $P<0.05$ ) and lactic acid bacteria ( $P<0.01$ ), 9.22 and 8.62 log CFU.g<sup>-1</sup> FM respectively. Whereas, lower ( $P<0.01$ ) number of molds and yeasts were detected in those prepared with addition of buffalo manure, 3.51 and 4.54 log CFU.g<sup>-1</sup> FM respectively. Significant increases were also observed in the total number of anaerobic bacteria and lactic acid bacteria with lower ( $P<0.01$ ) numbers of total aerobic bacteria due to substitution of urea with manure, however, lower ( $P<0.01$ ) number of molds and yeasts, 3.49 and 4.51 log CFU.g<sup>-1</sup> FM were detected in samples prepared with a combination of 100:0 of urea: manure.

**Keywords:** Rice straw, Manure, Silage, Urea, Microbes.

### **Introduction**

Nutritional requirements of ruminants are usually met through forages, characterized by low crude protein (CP), low energy, high fiber, and poor digestibility (Borquez *et al.*, 2009). The feeding value of poor quality fibrous feeds can be improved through various biological (ensiling), physical and chemical treatments (Sarwar *et al.*, 2002). Ensiling is a preservation method where some

bacteria break down cellulose and hemicellulose to simple sugars. Other bacteria ferment simple sugar to acidic end product under anaerobic conditions (Ogunjobi *et al.*, 2010). The production of organic acids lead to decrease pH and the ensiled materials are preserved (Weinberg & Muck, 1996). Thus, the main principles of preservation by ensiling are a rapid achievement of a low pH

by lactic acid fermentation and the maintenance of anaerobic conditions. The low pH in combination with anaerobic condition and un dissociated acids prevents growth of undesirable bacteria, molds, and yeasts (Scudamore & Livesey, 1998).

Silage microbes can basically be divided into two groups, namely the desirable and the undesirable. The desirable microbes are lactic acid bacteria. The undesirable ones are the organisms that can cause anaerobic spoilage (e.g., clostridia and enterobacteria) or aerobic spoilage e.g., yeasts, bacilli, listeria and molds (Ogunjobi *et al.*, 2010).

Additives are commonly used in ensiling, among these additives, urea has gained great attention as it increases the CP contents of roughages. Molasses, being a rich source of readily available carbohydrate, accelerates microbial activities which may cause rapid drop in pH (Nisa *et al.*, 2005).

Ruminant manure (M) is a valuable resource as a soil fertilizer providing nutrients required for the plant growth (Lazcano *et al.*, 2008). Traditionally manure is normally spread in the farm without any treatment. When applied in excess to the land requirement can lead to environmental pollution (Nasiru *et al.*, 2014).

To utilize manure as feedstuff different processing methods (dehydration, ensiling, pelleting, deep stacking and chemical preservation) have been employed (Borquez *et al.*, 2009; Sarwar *et al.*, 2011). Usage of manure as feedstuff in ruminant has the advantage of reducing pollution due to animal waste in addition to reducing feeding cost (Martinez-Avalos *et al.*, 1998). However, some factors have affected efficient utilization of manure including palatability, ease of handling, product quality and consumer

acceptance (Borquez *et al.*, 2009). The method commonly used in treating manure as feed resource is fermentation with soluble nitrogen source such as urea and carbohydrate such as molasses through silage (Sarwar *et al.*, 2006).

A manure silage was used to replace concentrates (Borquez *et al.*, 2010). Up to 30% manure inclusion in silage making was recommended for optimal utilization in ruminant feeding (Nasiru *et al.*, 2014).

Since ruminant manure is contaminated with many microbes, the current study aimed to investigate effect of ensiling urea-molasses treated rice straw (RS) on microbial community of fermented product.

## Materials & Methods

### Preparation of rice straw silage

This study was conducted in the Nutrition laboratory, Department of Animal Production to evaluate the microbial composition of rice straw silages prepared by substitution of urea with ruminant manure (sheep, cow, or buffalo) at different ratios. Rice straw was obtained from newly harvested rice field of Al-Najaf Province. Chemical and microbial composition of RS are shown in tables (1 & 2). Chemical composition was performed according to methods of AOAC (2004).

Samples of silage (control) were prepared by treating chopped rice straw (1-2 cm) with additives solution containing 10% of low quality date juice as a source of WSC and 2% of commercial urea as a source of nitrogen (N). Manure was dried in the field to reduce moisture content partially, and in the laboratory using air draft oven at 105 C° for 24 hrs. to ensure the termination of microbial potential (Hall & Keys, 1980). Urea was substituted with manure at 5 combinations of

**Table (1): Chemical composition of ensiled materials (%) on dry matter basis.**

Nutrients	Rice straw	Urea	Date juice	Manure		
				Sheep SM	Cow CM	Buffalo BM
DM	91.25	-	68.75	97.44	96.55	95.81
Ash	19.75	-	2.57	12.74	21.01	24.14
CP*	3.15	*287.5	2.20	7.81	4.35	4.14
EE	4.53	-	-	3.26	3.37	3.46
NDF	79.44	-	-	74.78	79.95	78.50
ADF	51.02	-	-	41.61	40.33	39.87
ADL	8.17	-	-	10.80	9.27	9.04
Cellulose	42.85	-	-	30.81	31.06	30.83
Hemicellulose	28.42	-	-	33.17	39.62	38.63
IVDMD (%)**	39.32	-	-	48.64	49.62	46.11
IVOMD (%)***	41.20	-	-	55.61	49.91	48.14

\* 46×6.25 \*\*IVDMD: in vitro dry matter digestibility and \*\*\*IVOMD: in vitro organic matter digestibility, were performed according to method described by Tilley & Terry (1963)

**Table (2): Microbial composition of straw and manure (log CFU.g<sup>-1</sup> DM).**

Microbes	Rice straw	Manure		
		Sheep, SM	Cow, CM	Buffalo, BM
Aerobic bacteria	6.3	5.6	5.84	5.47
Anaerobic bacteria	9.3	8.9	8.84	8.95
Molds	4	3.47	3.47	3.69
Yeasts	4.69	4.47	4.47	4.69
LAB	8.47	8.47	8.47	8.47

urea: manure, 90:10, 80:20, 70:30, 60:40 and 50:50. In control silage this combination was 100:0. All additives (urea, date juice, manure) were used on basis of dry matter (DM). Additives mix were diluted with quantity of water to ensure DM content of ensiled materials of about 40%. Additives solutions were sprayed on chopped RS with continuous mixing to distribute the solution to all parts of straw. Treated straw samples were then packed in double nylon bags to avoid deterioration of silage due to entering of air through probable punctures or cuts. Contents inside bags were squeezed by hands to exclude air may penetrated straw mass, closed tightly and stored in a bit silo for 60 days.

Rice straw silage (RSS) prepared with sheep (SM), cow (CM) or buffalo manure (BM) will be symbolized as RSS-SM, RSS-CM and RSS-BM respectively.

#### Microbial analysis

Microbial analysis of silages including total count of aerobic, anaerobic and lactic acid bacteria, yeasts and molds were performed in duplicate shortly after each bags were opened. one g was taken from each silage samples and blended with 9 ml of sterilized peptone water (Wu *et al.*, 2014). The suspensions produced were serially diluted ( $10^{-1}$  to  $10^{-10}$ ) in peptone water as described by Harrigan & McCane (1976). 100- $\mu$ L aliquots of each dilution was spread onto selective medias as follows:

Aerobic bacteria were counted on nutrient agar and autoclaved at 37°C for 24 hr (Sood, 1987). Plates were incubated in an incubator at 37°C for 24-48 hr. The same steps were performed to count anaerobic bacteria but plates were incubated anaerobically at 37°C for 24-48 hrs. (Adesoji *et al.*, 2010). Lactic acid bacteria (LAB) were counted on deMan, Rogosa, and Sharpe agar after incubation at 37°C for 48 hrs. under anaerobic conditions

(Addah *et al.*, 2014). Yeasts were counted on malt extract agar. Plates were incubated aerobically at 25°C for 72 h (Akintokun *et al.*, 2014). Molds were counted on potato dextrose. Plates were incubated aerobically at 25°C for 72 hrs. (Adesoji *et al.*, 2010).

Colonies were counted as viable numbers of microorganisms from plates containing a minimum of 30 and a maximum of 300 colonies. All analysis was carried out using the aseptic technique by using sterilized equipment and solutions to prevent contamination. Numbers of microbes are expressed as colony-forming units (CFU) per g of fresh silages (FM) and were log transformed.

### Statistical analysis

Data obtained were analyzed as a factorial experiment in completely randomized design by analysis of variance using Statistical Analysis System, (SAS, 2010).

### Results & Discussion

Microbial evaluation of rice straw silage included enumeration of total aerobic and anaerobic bacteria, molds, yeasts and lactic acid bacteria (LAB) according to critical role of these organisms in silage fermentation. Table (3) indicated numbers of microbial populations (log CFU.g<sup>-1</sup> fresh matter, FM) in reed silages as affected by addition of types of ruminant manure and urea: manure combinations.

As shown numbers of all microbes tested were significantly affected by types of manure. Statistical analysis revealed that there was a significant (P<0.05) increase in numbers of total aerobic bacteria in samples of RSS-SM as compared with RSS-CM and RSS-BM, (0.13 and 0.14 log CFU.g<sup>-1</sup> FM respectively). This increase may be caused by nature of SM and the way that silage samples

were prepared particularly, in relation with squeezing nylon bags to exclude air. Saeed *et al.* (2017) and Levital *et al.* (2009) considered anaerobic condition as a principal to ensure desired fermentation and producing good quality silage.

Regarding effect of urea: manure combinations, results showed that there was a significant (P<0.01) decrease in numbers of total aerobic bacteria in RSS samples prepared with each substitution of urea with manure. Numbers of this bacteria were 6.23, 5.95, 5.73, 5.76, 5.78 and 5.81 log CFU.g<sup>-1</sup> FM for urea: manure combinations of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 respectively. This decrease may be due to the better condition available to other silage microbes especially LAB, such a decrease in pH resulted from lowering urea level associated with introducing manure in these combinations. Urea is characterized with rapid degradation during ensiling (Sarwar *et al.*, 2006; Saeed *et al.*, 2017; Khan *et al.*, 2006). Moreover, increasing manure level in combinations may provide microbes with additional energy. Abid (2018) reported that substitution urea with ruminant manure not only decreased silage pH, but decreased ammonia nitrogen (NH<sub>3</sub>-N) and increased concentration of volatile fatty acids too.

Results exhibited that number of total anaerobic bacteria was increased (P<0.05) in samples of RSS-CM by 0.11 and 0.04 log CFU.g<sup>-1</sup> FM as compared with RSS-SM and RSS-BM respectively. This may be due to the improvement of ensiling conditions of RSS-CM samples thereby it was reflected on silage fermentation. Such positive changes in ensiling conditions were confirmed by Abid (2018).

Regarding the effect of urea: manure combinations, results revealed that there was a significant (P<0.01) increase in number of

**Table (3): Effect of type of ruminant manure and urea: manure combinations (%) on numbers of microbes in rice straw silage (log CFU\*.g<sup>-1</sup> FM ± SE).**

Microorganisms	Type of manure, TM			Urea: manure combinations (%), Com.						P-value	
	SM	CM	BM	100:0	90:10	80:20	70:30	60:40	50:50	TM	Com.
Total aerobic bacteria	5.97 <sup>a</sup> ± 0.04	5.84 <sup>b</sup> ±0.05	5.83 <sup>b</sup> ±0.05	6.23 <sup>a</sup> ±0.07	5.95 <sup>b</sup> ±0.03	5.73 <sup>c</sup> ± 0.05	5.76 <sup>c</sup> ±0.05	5.78 <sup>c</sup> ± 0.08	5.81 <sup>bc</sup> ±0.06	*	**
Total anaerobic bacteria	9.11 <sup>b</sup> ± 0.03	9.22 <sup>a</sup> ±0.03	9.18 <sup>b</sup> ±0.03	8.95 <sup>b</sup> ± 0.07	9.18 <sup>a</sup> ± 0.04	9.24 <sup>a</sup> ± 0.03	9.23 <sup>a</sup> ± 0.03	9.20 <sup>a</sup> ± 0.03	9.22 <sup>a</sup> ± 0.03	*	**
Molds	3.76 <sup>a</sup> ± 0.05	3.62 <sup>b</sup> ±0.03	3.51 <sup>c</sup> ± 0.01	3.49 <sup>d</sup> ± 0.03	3.56 <sup>cd</sup> ± 0.04	3.61 <sup>bc</sup> ±0.06	3.68 <sup>ab</sup> ± 0.07	3.66 <sup>bc</sup> ± 0.05	3.77 <sup>a</sup> ± 0.08	**	**
Yeasts	4.60 <sup>a</sup> ± 0.02	4.64 <sup>a</sup> ±0.03	4.54 <sup>b</sup> ±0.01	4.51 <sup>b</sup> ±0.02	4.51 <sup>b</sup> ± 0.02	4.54 <sup>b</sup> ± 0.03	4.64 <sup>a</sup> ± 0.02	4.66 <sup>a</sup> ± 0.06	4.71 <sup>a</sup> ± 0.01	**	**
LAB	8.60 <sup>a</sup> ±0.03	8.62 <sup>a</sup> ± 0.04	8.47 <sup>b</sup> ±0.01	8.32 <sup>c</sup> ± 0.00	8.53 <sup>b</sup> ± 0.03	8.54 <sup>b</sup> ±0.02	8.64 <sup>a</sup> ± 0.05	8.68 <sup>a</sup> ± 0.07	8.66 <sup>a</sup> ± 0.03	**	**

\* log colony forming unit/g FM

Means with different letters within each row are significantly differed at \* (P<0.05), \*\* (P<0.01)

total anaerobic bacteria in RSS samples prepared with inclusion of manure as compared with a urea: manure combination of 100:0. Numbers of these bacteria were, 9.18, 9.24, 9.23, 9.20 and 9.22 log CFU.g<sup>-1</sup> FM for 90:10, 80:20, 70:30, 60:40 and 50:50 combinations of urea: manure respectively. This can be explained on the basis of basic conditions associated with RSS samples prepared with urea only which may discourages growth of anaerobic microbes. Molds number of RSS-SM increased (P<0.01) as compared with RSS-CM and RSS-BM, (3.76, 3.62 and 3.51 log CFU.g<sup>-1</sup> FM respectively).

Results also revealed that there was a decrease (P<0.01) in molds numbers in RSS samples prepared with urea: Manure combination of 100:0 as compared with other combinations. This decrease may be attributed to the antifungal role of ammonia released from degradation of urea during ensiling (Kung *et al.*, 2000). This role seemed to be minimized with increasing level of urea in the urea: manure combinations used in preparing RSS in a current study. While molds were negatively affected by antifungal role of urea, it was significantly increased (P<0.01) as manure was introduced in combination of urea: manure due to lower NH<sub>3</sub>-N concentration (Abid, 2018).

Regarding yeasts, lower number (P<0.01) was detected in RSS-BM as compared with RSS-SM and RSS-CM, numbers of yeasts were 4.54, 4.60 and 4.64 log CFU.g<sup>-1</sup> FM respectively. Results of the current study indicated that yeasts number were also decreased (P<0.01) in RSS prepared with urea: manure combinations of 100:0, 90:10 and 80:20, numbers were, 4.51, 4.51 and 4.54 as compared with 4.64, 4.66 and 4.71 log CFU.g<sup>-1</sup> FM in RSS prepared with urea:

manure combinations of 70:30, 60:40 and 50:50 respectively. These results can also be explained on basis of antifungal role of ammonia (Kung *et al.*, 2000).

Numbers of lactic acid bacteria (LAB) were increased (P<0.01) in RSS-CM and RSS-SM as compared with RSS-BM, numbers were, 8.62, 8.60 and 8.47 log CFU.g<sup>-1</sup> FM respectively. Numbers of LAB were also affected by urea: manure combinations. Higher numbers (P<0.01) were detected in RSS prepared with combinations of 70:30, 60:40 and 50:50 as compared with other combinations. Numbers of LAB were, 8.64, 8.68 and 8.66 respectively, vs. 8.32, 8.53 and 8.53 log CFU.g<sup>-1</sup> FM for combinations of 100:0, 90:10 and 80:20 respectively. This result indicated that there was an increase in LAB numbers with increasing level of manure in those urea: Manure combinations. This increase may be due to availability of additional substrates particularly water soluble carbohydrates (WSC). Kuikui *et al.* (2014) referred to the importance of such nutrients (substrates) to maintain growth and activity of complex group of microbes particularly LAB which converts WSC to lactic acid (LA) and reduced pH accordingly.

Numbers of LAB can also be affected by nature of dominant LA fermentation, in homofermentative LAB lower WSC was consumed in comparison with heterofermentative (Weinberg & Muck, 1996), leading to more substrate available for LAB which may reflected positively on its numbers in silage.

Table (4) explained the effect of the interaction between type of manure and urea: manure combinations on numbers of microbes included in microbial evaluation of a current study. Statistical analysis showed that all microbes were affected (P<0.01) by that

**Table (4). Effect of interaction between type of manure and urea: manure combinations on numbers of microbes in rice straw silage (log CFU.g<sup>-1</sup> FM ± SE).**

Microbes	100% urea	Sheep manure					Cow manure					Buffalo manure					P- value
		Combinations of urea: manure					Combinations of urea: manure					Combinations of urea: manure					
		90:10	80:20	70:30	60:40	50:50	90:10	80:20	70:30	60:40	50:50	90:10	80:20	70:30	60:40	50:5	
Total aerobic bacteria	6.30 <sup>a</sup> ±0.02	5.90 <sup>bcdef</sup> ±0.04	5.89 <sup>bcdef</sup> ±0.07	5.94 <sup>bcde</sup> ±0.06	5.72 <sup>defg</sup> ±0.09	6.05 <sup>abc</sup> ±0.06	5.96 <sup>bcde</sup> ±0.06	5.49 <sup>g</sup> ±0.02	5.73 <sup>defg</sup> ±0.09	5.98 <sup>bcd</sup> ±0.02	5.75 <sup>cdefg</sup> ±0.02	5.99 <sup>bcd</sup> ±0.06	5.80 <sup>bcdef</sup> ±0.04	5.60 <sup>fg</sup> ±0.05	5.66 <sup>efg</sup> ±0.19	5.63 <sup>fg</sup> ±0.07	**
Total anaerobic bacteria	8.95 <sup>b</sup> ±0.13	8.96 <sup>b</sup> ±0.02	9.18 <sup>ab</sup> ±0.07	9.16 <sup>ab</sup> ±0.08	9.18 <sup>ab</sup> ±0.07	9.24 <sup>a</sup> ±0.06	9.30 <sup>a</sup> ±0.00	9.24 <sup>a</sup> ±0.06	9.30 <sup>a</sup> ±0.00	9.30 <sup>a</sup> ±0.00	9.24 <sup>a</sup> ±0.06	9.30 <sup>a</sup> ±0.06	9.30 <sup>a</sup> ±0.00	9.24 <sup>a</sup> ±0.06	9.12 <sup>ab</sup> ±0.07	9.18 <sup>ab</sup> ±0.07	*
Molds	3.49 <sup>d</sup> ±0.06	3.47 <sup>d</sup> ±0.00	3.91 <sup>b</sup> ±0.08	3.92 <sup>b</sup> ±0.13	3.56 <sup>cd</sup> ±0.05	4.20 <sup>a</sup> ±0.06	3.72 <sup>c</sup> ±0.08	3.47 <sup>d</sup> ±0.00	3.63 <sup>cd</sup> ±0.09	3.93 <sup>d</sup> ±0.06	3.47 <sup>d</sup> ±0.00	3.49 <sup>d</sup> ±0.02	3.47 <sup>d</sup> ±0.00	3.51 <sup>d</sup> ±0.04	3.48 <sup>d</sup> ±0.01	3.63 <sup>cd</sup> ±0.04	**
Yeast	4.51 <sup>cd</sup> ±0.04	4.47 <sup>d</sup> ±0.00	4.70 <sup>b</sup> ±0.07	4.71 <sup>b</sup> ±0.07	4.54 <sup>cd</sup> ±0.04	4.70 <sup>b</sup> ±0.01	4.54 <sup>cd</sup> ±0.04	4.47 <sup>d</sup> ±0.00	4.61 <sup>bc</sup> ±0.01	4.98 <sup>a</sup> ±0.08	4.73 <sup>b</sup> ±0.04	4.54 <sup>cd</sup> ±0.04	4.47 <sup>d</sup> ±0.00	4.60 <sup>bcd</sup> ±0.00	4.47 <sup>d</sup> ±0.00	4.70 <sup>b</sup> 0.01 ±	**
LAB	8.32 <sup>d</sup> ±0.01	8.54 <sup>c</sup> ±0.04	8.64 <sup>bc</sup> ±0.05	8.77 <sup>b</sup> ±0.10	8.55 <sup>c</sup> ±0.05	8.79 <sup>b</sup> ±0.06	8.60 <sup>c</sup> ±0.08	8.50 <sup>c</sup> ±0.03	8.64 <sup>bc</sup> ±0.09	9.04 <sup>a</sup> ±0.06	8.60 <sup>c</sup> ±0.02	8.47 <sup>cd</sup> ±0.00	8.47 <sup>cd</sup> ±0.00	8.51 <sup>c</sup> 0.04±	8.47 <sup>cd</sup> ±0.00	8.58 <sup>c</sup> ±0.04	**

\* log colony forming unit/g FM

Means with different letters within each row are significantly differed at \* (P&lt;0.05), \*\* (P&lt;0.01).

interaction. Higher numbers ( $P < 0.01$ ) of total aerobic bacteria (6.30) were detected in samples of RSS prepared with urea: manure combination of 100:0 (No manure), whereas, lower numbers ( $5.49 \log \text{CFU.g}^{-1} \text{FM}$ ) were associated with RSS-CM samples prepared with urea: manure combination of 80:20. This result can be explained on the basis of availability of suitable medium for other microbes particularly LAB such as low pH resulted from reduced level of urea characterized with rapid degradation during ensiling (Khan *et al.*, 2006; Sarwar *et al.*, 2006; Saeed *et al.*, 2017). Consistently, higher numbers ( $P < 0.01$ ) of total anaerobic bacteria including LAB were detected in all RSS samples prepared with addition of manure regardless to its type (SM, CM or BM).

Results of interaction effect also explained that higher numbers ( $P < 0.01$ ) of molds were detected in RSS-SM samples prepared with urea: Manure combination of 50: 50 (4.20) as compared with lower numbers detected in RSS-SM samples prepared with a combination of 90: 10 (3.47), RSS-CM prepared with a combination of 80:20 (3.47) and 50:50 (3.47) and RSS-BM prepared with a combination of 80:20 ( $3.47 \log \text{CFU.g}^{-1} \text{FM}$ ). This may due to antifungal effect of ammonia (Kung *et al.*, 2000) produced during ensiling from rapid degradation of urea (Catchpoole, 1970).

Higher numbers ( $P < 0.01$ ) of yeast (4.98) was observed in RSS-CM samples prepared with urea: Manure combination of 60:40 as compared with  $4.47 \log \text{CFU.g}^{-1} \text{FM}$  detected in RSS-SM prepared with a combination of 90:10, RSS-CM prepared with a combination of 80:20 and RSS-BM prepared with combinations of 80:20 and 60:40. This result may attributed to the role of ammonia accumulated during ensiling.

Numbers of LAB were higher ( $P < 0.01$ ) in RSS-CM samples prepared with urea: manure combination of 60:40 (9.04) as compared with other samples. Whereas, lower numbers were detected in RSS prepared without addition of manure (combination 100:0) and in RSS-BM samples prepared with combinations of 90:10, 80:20 and 60:40, numbers of LAB in these RSS were 8.32, 8.47, 8.47 and  $8.47 \log \text{CFU.g}^{-1} \text{FM}$  respectively. The priority of RSS-CM prepared with a combination of 60:40 may be associated with better condition for LAB growth. McDonald *et al.* (1991) reported that acidic medium in the initial phases of ensiling is a cornerstone for the subsequent dominance of LAB on silage fermentation. Abid (2018) attributed higher LAB numbers in RSS-CM to a decrease in pH.

## Conclusions

The results suggest that the substitution of urea with ruminant manure at different levels had clear effects on microbial counts of rice straw silages. Changes in numbers of total aerobic, anaerobic bacteria and LAB referred to an improvement in silage quality. Anaerobic condition associated with preparing silage samples may have a significant influence on silage fermentation. Additional efforts should be done to overcome the increase in numbers of undesired microbes such as molds and yeasts.

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