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Study on Growth Rate Performance of Sheep Fed with Super Napier Grass Silage Treated with *Lactobacillus Buchneri* and *Lactobacillus Plantarum*

By Noemi C. Liangco, Joel L. Reyes, Estrelita M. Pascua, Virapol Jamsawat, Chongko Seatung & Vorrapol Jamsawat
Isabella State University

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STUDYONGROWTHRATEPERFORMANCEOFSHEEPFEDWITHSUPERNAPIERGRASSSILAGETREATEDWITHLACTOBACILLUSBUCHNERIANDLACTOBACILLUSPLANTARUM

Strictly as per the compliance and regulations of:

Study on Growth Rate Performance of Sheep Fed with Super Napier Grass Silage Treated with *Lactobacillus Buchneri* and *Lactobacillus Plantarum*

Noemi C. Liangco [α], Joel L. Reyes [σ], Estrelita M. Pascua [ρ], Virapol Jamsawat [Ѡ], Chongko Seatung [¥] & Vorrapol Jamsawat [§]

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The effect on the growth rate performance of sheep were measured and analyzed by using analysis of variance in a completely randomized design (CRD).

The results revealed that super Napier grass (SNG) silages treated with inoculants had a higher levels of crude protein, crude fiber, crude fat, ash and nutrient detergent fiber compared with the untreated SNG silages. The DM fraction of the SNG treated silages was increased in contrast to the untreated where the moisture content increased. The experiment trial indicated that SNG silage treated with *Lactobacillus buchneri* and *Lactobacillus plantarum*(T2 and T3) influenced. In terms of the growth parameters, significant (P<0.01) differences were noted on body weight, weight gained, average daily gain (ADG) feed consumption and feed efficiency (FCR). The feeding trial indicated that SNG silage treated with *Lactobacillus buchneri*and*Lactobacillus plantarum* (T2 and T3) influenced sheep growth rate performance, weight gain, feed intake and days to market. Thus, addition of beneficial microbes improve the nutritional quality of silage and increased nutrients levels resulting to higher growth of sheep.

Keywords: growth rate performance, super napier grass silage, lactobacillus buchneri and lactobacillus plantarum.

I. Introduction

Feed resources for ruminant livestock production in the country(Philippines and Thailand)normally are natural forage crops, natural pastures and natural plants but almost are low in quality of grasses which are limited in supply during the dry season. Today, ruminant animals are now fed with fermented or preserve feeds and has been very popular especially with dairy and beef cattle that require high level of nutrition in order to achieve high milk and beef production. Nowadays, the use of corn silage and others fodder crops as green forage in ruminant feeding has increased rapidly due to its high yielding properties, relatively high content of energy, palatability and easy incorporation in total mixed ration. Scarcity of feed for ruminants is one of the important problems for rearing livestock during summer especially in the country and other tropical countries (Jamsawat, 2017). However, livestock raisers can conserve feed resources by producing silages when feed resources are abundant during rainy season. Since silage is an alternative for ruminants especially in production situations that require consistent nutrition on a daily basis, condition of silage has a significant impact on its quality for reasons that forage often contains many detrimental types of bacteria. In fact, the primary goal of making silage is to maximize the preservation of original nutritional value of the forage crop at the highest value possible during storage for feeding at a later date. The traditional method of fermentation in the silo however, is a much uncontrolled process usually leading to less than optimal preservation of nutrients.

Feed shortage and low quality of forage crops in the country are the major constraints to the development of ruminant industry. To overcome these problems, feeding of ruminants with conserved forages which is becoming popular among enterprising livestock raisers in the country is an important feeding strategy to ensure the success of ruminant production in the country. (Khaini et al., 2015). Small ruminant production is a very significant component of livestock production throughout the world and more specifically in the developing countries. Small ruminants has the ability not only to survive different environmental conditions but also able to utilize poor quality feed.

In recent times, the use of corn silage and others fodder crops silage as green forage in ruminant

Author α σ ρ: Faculty of Agriculture, Isabella State University, Philippines.
Author Ѡ ¥: Faculty of Agriculture and Natural Resources.
Author §: Faculty of Humanity and social Science, Rajamangala University of Technology Tawan-Ok, Thailand.
e-mail: virapolj@yahoo.com

feeding has increased rapidly due to its high yielding properties, relatively high content of energy, palatability and easy incorporation in total mixed ration. The concept of adding a microbial inoculant to silage was to add fast-growing homo fermentative lactic acid bacteria (LAB) in order to dominate the fermentation resulting in a higher quality silage, (Kung and Ranjit, 2001). *Lactobacillus buchneri* and *Lactobacillus plantarum* are some of the most common LAB inoculants in the fermentation of silage. Poorly preserved silages have poor fermentation quality; they are unpalatable to stock and reduced feed intake. These silages are also likely to have suffered extensive degradation of protein, resulting in poor utilization of the silage nitrogen by animals. In order to assist in the fermentation process, various silage additives have been used to improve the nutrient and energy recovery in silage, and when fed to livestock it will subsequently improve animal performances. The different species of lactobacillus were tested to find out the effectiveness of the lactobacillus species as additives in ensiling "super Napier" known as "Pakchong 1" which was developed and produced in Thailand. The "Super Napier Grass" (SNG) is a cross of ordinary Napier grass (*Pennisetum purpureum*) and Pearl Millet (*Pennisetum glaucum*) can yields more crude protein of about 16 to 18 percent (Kiyothong, 2014). This grass requires lower inputs and easier to establish compared to corn and can be a good alternative, especially in production situations that require consistent nutrition on a daily basis. It is for this reason that two silage additives were tested to find out the effectiveness of preserving the quality of super Napier grass and its effect on the growth rate performance of sheep.

II. Methodology

a) *Silaging and Evaluation*

The *L. buchneri* and *L. plantarum* additives that were used in this experiment were purchased in Korean Culture Collection of Microorganism. Cultures that were cultivated an aerobically in De Man, Rogasa and Sharpe (MRS) agar medium in 250 ml flasks incubated at 30°C for 2 day in an orbital shaker(Thermo Scientific Max Q 2000, USA) at 100 rpm. Cultures were diluted in demineralized water before use. Inoculant wadded at a theoretical rate 1.0x10^5cfu/g. prior to inoculation, inoculant will be diluted with distilled water to achieve the required concentration and keep for silage production.

Super Napier grass were sourced out from Isabella State University, Enchague, Isabella farm. The SNG were manually harvested at the maturing stage approximately 80 to 90 day of re growth and were chopped into 2-3 centimeters.

Fifteen plastic drum silos with a capacity of 20 L were randomly assigned to three treatments by four factor experiment. The SNG were ensiled into 20-L drum silo and stored in dark and ambient temperature(5°C - 10°C)for 0, 7, 15 and 30 days. The treatments were the following:

Treatment 1- Super Napier grass without inoculants(control).

Treatment 2- Super Napier grass treated with *Lactobacillus buchneri.*

Treatment 3- Super Napier grass treated30°C with *Lactobacillus plantarum.*

b) *Ensiling Procedure for Super Napier Grass*

Fifteen kilogram of SNG grass each replication were inoculated with or without 3% (w/v) of *L. buchneri* or *L. plantarum* through spray method followed by thorough mixing. The samples were ensiled into 20-L drum silo and stored in dark and ambient temperature for 0, 7, 15, and 30 days. Triplicate silos were opened and the upper part 1/5 of silages were discarded before sampling of approximately 100 g. after each incubation period. Silage extracts will be prepared immediately by macerating a 50 g. silage samples with a 300 ml. of distilled water. These were collected through double cheesecloth and used to determine pH value and concentrations of volatile fatty acids (VFA) and volatile basic nitrogen and ethanol. Dry matter (DM) content of grass and silages were determined by a vacuum freeze-drying method (Uchida, 1986). The dried samples were grinded and then the crude protein was determined by the Kjeldahl method. NDF, ADF, and ADL were measured by the method of Goering and Van Soest (1970). Water soluble extracts was prepared by macerating 40 g of fresh silage sample in 400ml distilled water. The pH of the extracts were measured by using electric pH meter (PH71/PH72 personal pH / ORP meter, Yokogawa Electric Corporation, Japan). Fermentation products, pH and ammonia were determined in silage extracts, prepared by adding 270g demineralized water to 30g silage and homogenizing for 5min in a laboratory blender. Volatile fatty acids were analyzed using an HPLC device (Agilent Technologies 1200 series).

c) *Data Gathered and Statistical Analysis*

The chemical analysis of SNG and the chemical analysis of super Napier grass silage were determined, recorded and served as basis of evaluating the quality of silages as affected by the different additives/inoculants. All data gathered were tabulated and analyzed using analysis of variance in Completely Randomized Design (CRD). Significant differences among treatments were also analyzed using the Least Significant Difference (LSD).

d) *Growth Rate Performance of Sheep*

A total of thirty (30) heads of growing sheep were acquired from a commercial farm in Bulacan and

Nueva Ecija, Philippines. The animals were randomly distributed into three (3) treatments. There were ten (10) animals assigned for each treatment each serves as replication. The experiment was laid out using the Completely Randomized Design (CRD) with the following treatments:

Treatment 1- Super Napier grass silage without inoculants (control).

Treatment 2- Super Napier grass silage with *L. buchneri*.

Treatment 3- Super Napier grass silage with *L. plantarum.*

e) Feeding and Management of Experimental Animals

The sheep were gradually introduce to their respective diet over two week's period and were fed ad libitum before the feeding trial officially began. Each experimental sheep were given the assigned diet throughout the feeding period. The amount of silage offered daily is computed approximately 4-5 % of body weight on a dry matter basis. The total amount of daily feed required were divided into morning (8:00) and afternoon (17:00) feeding. All animals were given free access to fresh drinking water and trace mineral salts throughout the experiment. The experimental sheep were permitted to adapt to the diets for two weeks prior to the actual feeding trial. Initial weights of the animals were measured after the acclimatization period. Clean and fresh drinking water were given at all times. The water was changed two times a day, morning and afternoon or as needed. Identical care and management were provided to the sheep throughout the feeding trial.

f) Weighing of Experimental Animals

The initial weight were recorded. Bi-weekly weighing were done during the entire observation period in the morning before feeding every times. The final body weights were determined and blood samples were collected at the end of the feeding trial. The experimental animals were weighed before feeding in the morning. All data gathered on the growth rate performance parameters of sheep were recorded, evaluated and analyzed using analysis of variance in Completely Randomized Design (CRD). Significant differences among treatments were also analyzed using the Least Significant Difference (LSD).

III. Results and Discussion

a) Chemical Analysis of Super Napier Grass

The chemical analysis of SNG was shown in Table 1. Results of the chemical analysis of super Napier grass as fresh and as dry matter bases were analyzed. The basis analysis were as follows: crude protein content was 1.21 %, crude fiber, 5.37%, crude fat, 10.12%, moisture, 85.09 %, ash, 1.01% and neutral detergent fiber, 10.20 percent. Likewise, the analysis as dry matter basis were as follows: crude protein content, 8.12%, crude fiber, 36.02%, crude fat, 67.87%, ash, 6.87% and neutral detergent fiber, 68.41%.

Table 1: Chemical analysis of super Napier grass

Parameters	Fresh	Dry Matter
Crude Protein, %	1.21	8.12
Crude Fiber, %	5.37	36.02
Crude Fat, %	10.12	67.87
Moisture, %	85.09	-
Ash, %	1.01	6.77
Neutral Detergent Fiber, %	10.20	68.41

The chemical analysis used in this study is below the findings of percent (Kiyothong 2014) with a CP. concentration of 16−18 percent of a 45 days cutting interval. The low CP. concentrations of Napier grass was attributed to the high structural cell wall carbohydrates that increase rapidly with maturity causing decline in CP. concentration and digestibility (Van Soest 1994). Likewise, studies of (Cuomo et al., 1996), also demonstrated the effects of cutting interval on yield and quality vary with cultivars management practices and environmental conditions. Therefore, appropriate cutting management is essential for high production and quality of this species (Tessema et al., 2010).

b) Chemical Analysis of Untreated and Treated Super Napier Grass Silage

The chemical analysis(composition) of SNG treated and untreated (DM basis) was shown in Table 2. Results of the analysis of the different silages treated and untreated varied among the treatments. After 7 days of fermentation, it was observed that the level of crude protein content of silage treated with inoculants had increased by 20% in T2 and 16% in T3, while those silage treated with plain water, (T1) had a reduction of 19.17 percent. Similar observation of improvement with silages treated with inoculant was noted on crude fiber, ash and neutral detergent fiber. The pH level prior to ensiling ranged from 6.5 to 7.0 and at the end of 30 days fermentation, the pH level drop due to lactic acid production with pH ranged from 4 to 4.5. The variation on the pH levels was probably due to the different inoculants used. It is worthy to mention that at the end of the 30th day of fermentation nutrient levels was improved. There was a noticeable improvement of ash and crude fiber content in all treatments, treated or untreated. Although there was a slight reduction on the crude protein content of SNG in all treatments from the start, the crude protein content of the treated silages were higher than the untreated ones. On the other hand, there was a substantial increase of crude fat in the untreated silages compared to the treated silages; however, at the end of 30 days of fermentation, there was a marked increase in the level of crude fat in treated silages higher than the untreated silages.

Table 2: Chemical composition of untreated and treated SNG silage with bacterial inoculant at DM basis

Parameters	T1 – w/o inoculant		T2 - *Lacto. buchneri*		T3 - *Lacto. Plantarum*	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Crude Protein, %	2.66	2.15	1.72	2.07	2.24	2.59
Crude Fiber, %	5.79	5.17	5.23	6.00	5.17	6.92
Crude Fat, %	1.09	13.55	2.47	1.39	8.88	1.29
Ash, %	2.28	1.93	1.93	2.21	1.51	2.09
NDF, %	11.69	10.31	11.96	13.06	10.84	12.56
Dry Matter, %	82.05	84.33	83.17	81.08	83.93	80.90

The result of the study conforms to the main objective of manufacturing silages which is to maximize the preservation of original nutrients in the forage crop for feeding at a later date. The result likewise confirmed reports that fermentation is really an uncontrolled process usually leading to less than optimal preservation of nutrients. The used of inoculants is therefore necessary to assist in the fermentation process. Silage additives have been used to improve the nutrient and energy recovery in silage, and when fed to livestock it will subsequently improve animal performances. In conclusion, these studies confirmed that the applying of molasses improved fermentative quality, feed intake and digestibility of Napier grass (Bureenok, etal., 2012).

The graphical presentation of the untreated and treated super Napier grass is illustrated in figure 1, 2 and 3. The moisture content in T1- control increases as fermentation progresses then dropped starting on the 15th day to 30th day of fermentation. Whereas, the treated silages decreases consistently from day 7 to day 30 of fermentation. It is interesting to note that there were clear differences on the nutrient levels among the different treatments with higher levels in the treated silages. The illustration is a clear indication that nutrient levels in silaging is improved and preserved as manifested by the absence of mold. The result of this study is supported by Driehuis and Wikselaar (2000) in corn silage treated with *L. buchneri* was more stable than untreated silage. They suggest that improved aerobic stability was due to the ability of *L.buchneri* to ferment lactic acid to acetic acid and 1, 2 propanediol.

Although the result are encouraging, it should be noted that other literature reports varied markedly among due to environmental factors. The variability in results from this experiments involving silage fermentation indicates that further evaluations are necessary to broaden the database of additives for the ensilage of super Napier grass.

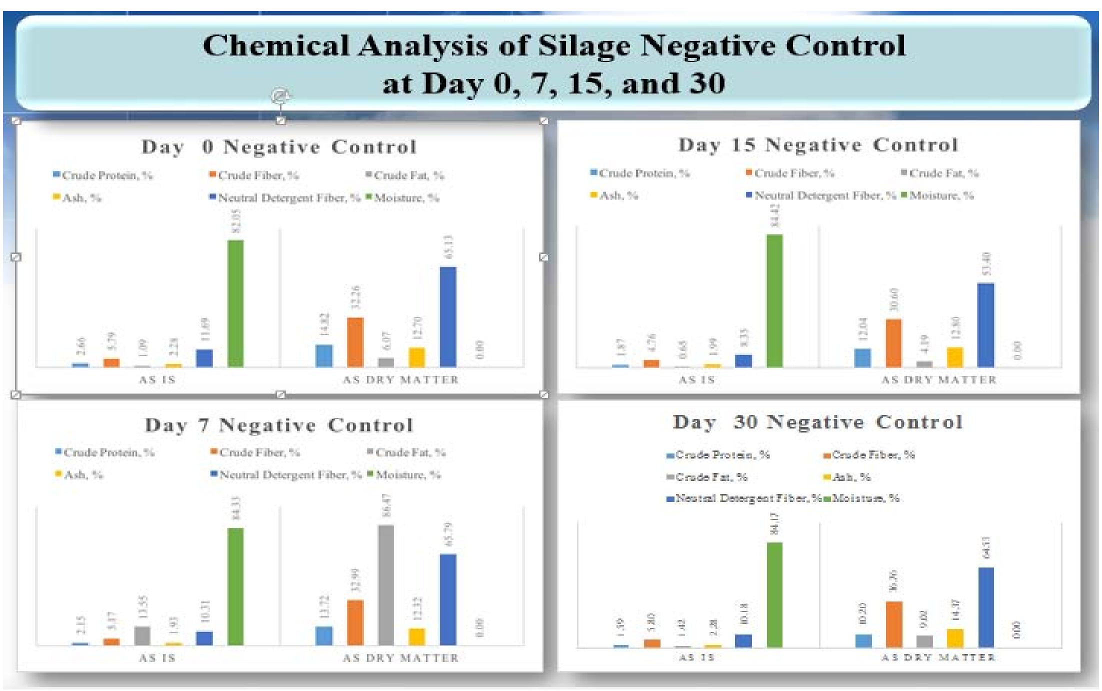

Figure 1: Graphical Presentation of Super Napier Grass Silage Treated with Plain Water (control) from day 0 to day 30 of Fermentation

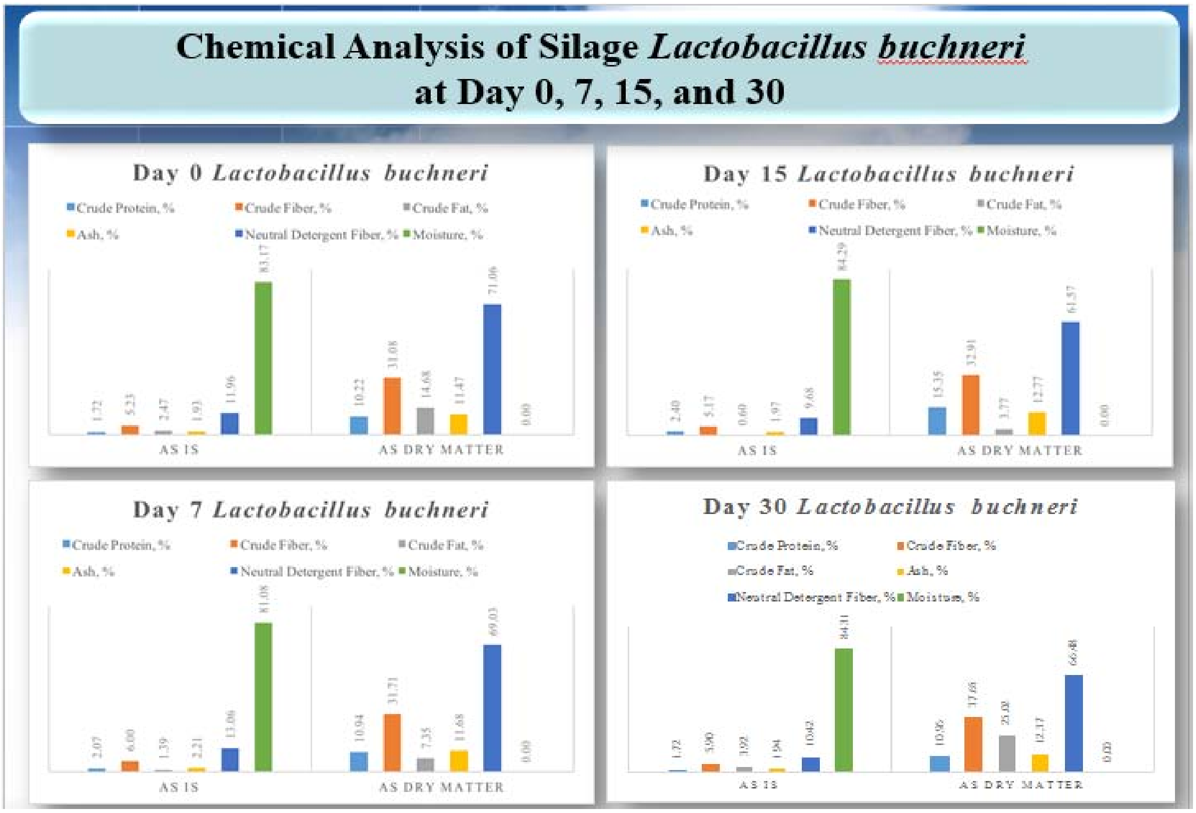

Figure 2: Graphical Presentation of Super Napier Grass Silage Treated with *L. buchneri* from day 0 to day 30 of Fermentation

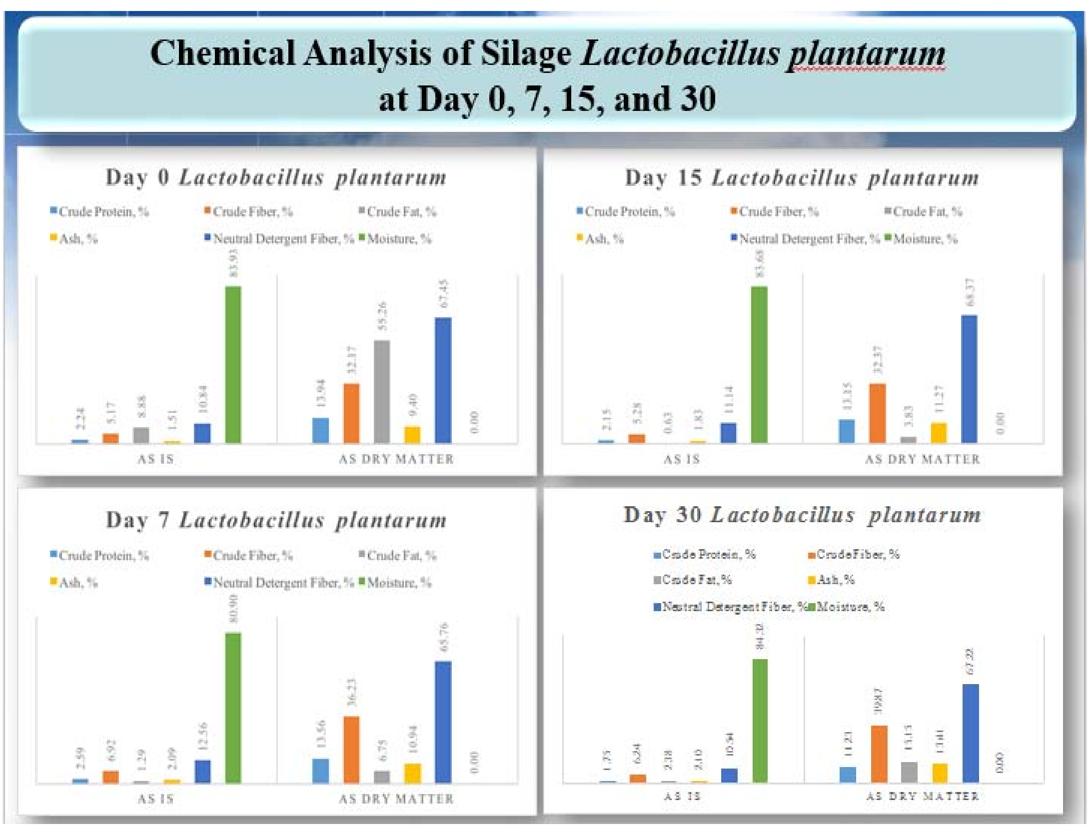

Figure 3: Graphical Presentation of Super Napier Grass Silage Treated with *L. Plantarum* from day 0 to day 30 of Fermentation

c) Growth Rate Performance of Sheep

The health and vigor of the experimental sheep were generally normal throughout the duration of the feeding trial. No apparent signs or symptoms of diseases were observed.

d) Body Weight

Significant differences were noted on the body weight of the experimental sheep (Table 3 and figure 4). At the end of the 42nd day of feeding, significant differences ($P<0.05$) was noted among the different treatments. Treatment 2 and 3 significantly had a higher BW over Treatment 1 (control) but no significant difference was observed between Treatment 2 and 3. Though similar growth pattern was observed, the result at the end of the 56th day of feeding revealed that Treatment 3 obtained the heaviest BW that is significantly heavier than T2 and T1 (control).

At the end (70th day) of the feeding trial, Treatment 3 with *Lactobacillus buchneri* was observed to be significantly heavier ($P<0.05$) than Treatment 2 with *Lactobacillus plantarum* and 1 (control without additives). Although Treatment 2 and 1 were statistically the same, there is a clear indication that sheep fed SNG treated silage (T2) is numerically higher than the sheep fed with untreated silage (T1). The higher BW of sheep fed with SNG treated silage might be due to the additives that could have improved digestibility resulted to higher nutrient intake as the result to rapid rate of fermentation occurring in the rumen This observation is supported by the findings of Kung and Ranjit (2001) that animals respond positively to microbial inoculants for ensiling in terms of intake, gain, and milk production.

Table 3: Initial Body Weight and Bi-Weekly Body Weight of Sheep Fed with Silage

Treatments	Ave. Bi-weekly Body Weight (BW), kg.					
	Initial	0-14	15-28	29-42	43-56	57-70
1- Control	16.50	17.05	17.95	19.30$_b$	20.05$_b$	20.75$_b$
2 - *Lactobacillus buchneri*	16.00	17.20	18.25	20.35$_a$	20.85$_b$	21.70$_b$
3 - *Lactobacillus plantarum*	16.25	17.50	19.20	20.75$_a$	21.55$_a$	22.05$_a$
Result	ns	ns	ns	*	*	*
% C.V.	6.09	5.67	5.23	4.02	3.83	3.51

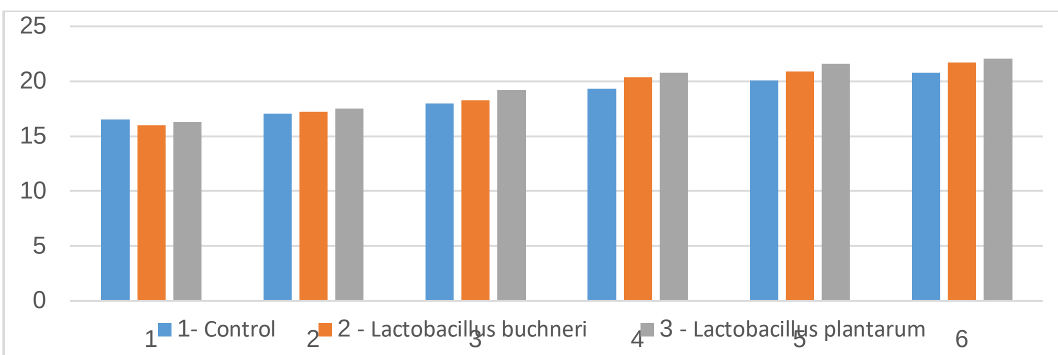

Figure 4: Graphical Presentation on Bi-weekly Body Weight of Sheep Fed with Super Napier Grass Silage

Result of the study showed that feeding of super Napier grass silage washigher than the sheep fed with untreated silage as similar with the studies conducted by Khaini et al., (2015) in cattle reported that feeding of silage influenced steer growth rate. The result of the study on the feeding of silage to sheep was supported by the studies of Kung and Ranjit (2001) with corn silage as the main source of feed for cattle but also as a combination with other forages including pasture grass. Studies on the effect of lactic acid bacteria (LAB) on animal performance indicated that feeding cattle with silages treated with LABs improve ruminant performance. Likewise, in several trials conducted by Muck, (1993) reported that inoculants exhibited substantial effect on performance on live weight gain, milk production, increase in intake and feed efficiency. This suggest the ensiling of SNG with microbial inoculants to improve the nutritional quality of SNG especially when there is abundant supply of grasses and feeding them to sheep during summer time when there is scarcity of roughages.

e) Feed Consumption

The average bi-weekly DM intake of silage of the experimental animals is shown in Table 4 and graphically presented in Figure 5. Non-significant result was observed on the DM intake of silage on the early stages(14th to 28th) of the experiment but results showed significant variation ($P<0.05$) as noted on the 29th to 42th day of feeding period but from 56th to 70th day of feeding, significant differences ($P<0.01$) were noted among the experimental animals. The cumulative feed consumption likewise showed significant ($P<0.01$) differences among the treatments.

Based on the result of the feeding trial, the DMI increased linearly with the silage containing inoculants. This could be due to the higher palatability and good fermentation characteristics of feeds which attracted the sheep to consume more amount of SNG silage. Another reason for the increased in the DM intake of the feed might be due to the chemical composition of the SNG silage and probably due to higher amount of fermentable carbohydrate and energy which increases the digestibility of the SNG silage with inoculants. Likewise, the DM intake differences may be attributed to rapid rate of fermentation occurring in the rumen. In a study conducted by Wiese et al., (2003) on the growth and carcass characteristics of prime lambs fed diets containing urea, lupines or canola meal as a crude protein source, he reported that higher DMI was due to a better availability of nutrients which are readily been degraded by rumen microbes.

Table 4: Average Bi-weekly and Total Dry Matter Intake of experimental animals (kg.)

Treatments	Average Bi-weekly Feed Consumption (Dry Matter), kg.					
	0-14	15-28	29-42	43-56	57-70	Total
1- Control	8.51	9.05	9.70b	10.05bc	10.40b	47.71[b]
2 - *Lactobacillus buchneri*	8.70	9.65	10.15b	10.70ab	11.15a	50.35[b]
3- *Lactobacillus plantarum*	8.80	10.00	10.35a	10.80a	11.25a	51.20[a]
Result	ns	ns	*	**	**	*
% C.V.	6.18	6.18	4.12	3.43	6.09	3.98

Means with common letter are not significantly different with each other using LSD

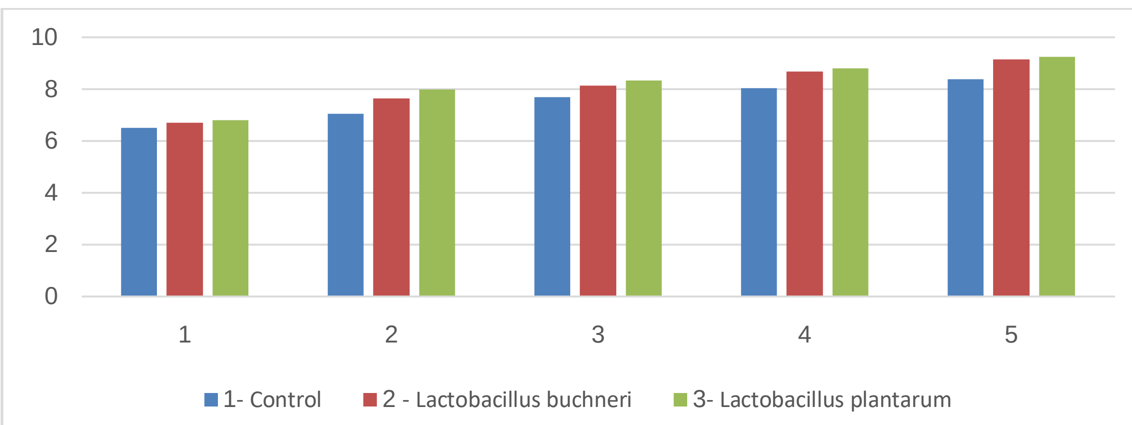

Figure 5: Graphical Presentation on Bi-weekly Feed Consumption (DM) of Sheep Fed with Super Napier Grass Silage

f) Body Weight Gain, Average Daily Gain, DM Intake and Feed Conversion Ratio

Table 5 present the total body weight (BW)gain, average daily gain, dry matter intake and feed conversion ratio of sheep fed silage diet during the 70 days of feeding trial. Significant differences ($P<0.01$) were observed among treatments on the total BW gain of sheep fed with SNG Silage treated with additives. The average daily gain (ADG) as a measure for growth also revealed significant ($P<0.01$) differences among the treatments. The positive improvement of Treatment 2 and 3 was the result of higher nutritive values of silages with additives as compared to the silages without additives. This implies that *Lactobacillus buchneri* and *Lactobacillus plantarum* (T2 and T3) additives can improve quality of silages that can provide higher nutritive value and therefore influence growth rate of sheep.

Sheep fed with SNG silage diet treated with *Lactobacillus plantarum* additives (T3) was the most efficient feed converter and this could be due to higher levels of nutrients as reflected in the chemical composition of silages at 7days of treatment.

Table 5: Average Body Weight Gain (ABW), Average Daily Gain (ADG), Dry Matter (DM) intake, and Feed Conversion Rate (FCR) of sheep fed with SNG silage

Treatments	Ave. Bi- weekly Body Weight (BW) Gain, kg			
	Body Wt. Gain (Kg.)	ADG, Grams	DM intake (Kg.)	FCR (DM)
1- Control	4.25b	60.72b	47.71^{b}	8.94b
2 – *Lacto. buchneri*	5.60a	80.00a	50.35^{b}	7.26ab
3- *Lacto. plantarum*	5.80a	82.85a	51.20^{a}	7.21a
Result	**	**	*	**
%C.V.	3.98	6.50	5.79	10.24

Means with common letter are not significantly different with each other using LSD

The result of the study on the feeding of silage to sheep was also supported by the studies of Kung and Shaver (2001) with corn silage as the main source of feed for cattle but also as a combination with other forages including pasture grass. Weinberg (2013) described the effect of lactic acid bacteria (LAB) on animal performance that feeding cattle with silages treated with LABs improve ruminant performance. Likewise, in several trials conducted by Spoelstra(1991) and Muck(1993) reported that inoculants exhibited substantial effect on performance on live weight gain, milk production, increase in intake and feed efficiency. Result of the study was similar with the studies conducted by Khani et al.,(2015) in cattle that feeding of silage influenced steer growth rate. This suggest the ensiling of SNG with microbial inoculants to improve the nutritional quality of SNG especially when there is abundant supply of grasses and feeding them to sheep during summer time when there is scarcity of roughages. In conclusion, these studies confirmed that the applying of inoculants improved fermentative quality, feed intake and digestibility of Napier grass (Bureenok, etal., 2012).

IV. Conclusion and Recommendation

The trial revealed that there is a great potential for improvement with the addition of beneficial microbes such *Lactobacillus buchneri* and *Lactobacillus plantarum* as it improves nutritional quality of SNG silage thus influenced ruminant animals (sheep) growth rate performance, average daily gain (ADG),DM feed intake and feed efficiency.

It is recommended that more research is needed to broaden the database of additives for the ensilage of SNG and to determine the nutrient digestibility and the combination of grasses to silage to reduce feed costs. Likewise, there is a need to find out if there is a deleterious effect to the end product's taste, tenderness, palatability and overall acceptability of mutton. However more research is needed to elucidate the mode of action of SNG treated silages.

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