JAPR

Vol. 02, Issue, 01, pp. 1-7, 8 *February*, **2025**

RESEARCH ARTICLE

Effects of Lactic Acid Bacteria (LAB) Inoculants on the Fermentation Quality of King Grass Silage Over Different Ensiling Periods

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ARTICLE INFO

Article History:
Received 15 *January*, 2025
Received in revised form 30 *January*, 2025
Accepted 3 *February*, 2025
Published online 8 *February*, 2025

Keywords:
Fermentation Quality
Ensiling day
Lactic Acid Bacteria
King Grass
Silage

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ABSTRACT

This study assessed how King Grass (*Pennisetum purpureophoides*)'s ensiling qualities and chemical makeup were affected by lactic acid bacteria (LAB). Four treatments were used: a control (KC) with 2 mL/kg of sterilized water, commercial *Lactobacillus plantarum* (KP), *L. plantarum* isolated from Napier Grass (KN), and *L. plantarum* isolated from Italian Ryegrass (KI). Lactic acid (LA), ethanol, and propionic acid (PA) levels in LAB-treated silages were significantly higher ($P<0.05$) than in the control. Nevertheless, there was no discernible difference ($P>0.05$) in dry matter (DM) or PA between treatments at the conclusion of the ensiling period. Acetic acid (AA) and ammonia nitrogen/total nitrogen (NH_3-N/TN) decreased ($P<0.05$), although LA and ethanol increased significantly ($P<0.05$). LAB, mold, and yeast populations also showed significant changes ($P<0.05$).

1. Introduction

Ensiling is a widely used preservation method for forage, allowing for year-round feed availability while maintaining nutritional quality [1]. The success of ensiling depends on rapid acidification, mainly driven by lactic acid bacteria (LAB), which inhibit spoilage microorganisms and enhance silage stability [2]. King grass (*Pennisetum purpureum* × *P. americanum*), a high-yielding tropical forage, is commonly ensiled to improve its digestibility and nutrient retention for ruminants [3]. However, due to its high moisture content and low water-soluble carbohydrate (WSC) levels, its fermentation process often requires optimization to achieve desirable silage characteristics [2,3]. LAB inoculants are commonly used as silage additives to enhance fermentation efficiency by accelerating lactic acid production, reducing pH, and inhibiting undesirable microbes [4]. The effects of LAB inoculation on silage quality can vary depending on factors such as the bacterial strain used, forage composition, and the duration of ensiling [5]. Different ensiling periods influence the microbial succession and fermentation

dynamics, ultimately affecting the nutritional value, aerobic stability, and digestibility of silage [6]. This study aims to evaluate the effects of LAB inoculants on the fermentation quality of King grass silage over different ensiling periods. By analyzing key parameters such as pH, organic acid profiles, microbial populations, and nutrient composition, this research will provide insights into the role of LAB in improving silage preservation and optimizing feeding value for livestock.

2. Materials and methods

2.1. Silage Preparation

At the mid-growth stage, King Grass (*Pennisetum purpureophoides*) was gathered from Nanjing Agricultural University's experimental grassland in China. Using a chopper, the grass was cut into 1- to 2-cm pieces before being placed in five-liter anaerobic PET bottles. One of the following treatments was applied to each container, which held 3.2 kg of fresh King Grass: Ecosyl Products Inc., USA's *Lactobacillus plantarum* (MTD/1CB) KP; Napier Grass isolation of KN- *L. plantarum*; Isolation of KI- *L. plantarum* from Italian Ryegrass; KC: Control (no injection of microorganisms). A concentration of 1×10^6 CFU/g was applied to each type of bacteria. Ten samples from each treatment were kept at room temperature after being wrapped in plastic tape. On days 14, 30, and 60, silage was opened for examination.

2.2 Chemical Analysis

AOAC [7] methods were used to determine the dry matter (DM) and crude protein (CP) contents of both fresh and ensiled samples. The anthrone reagent was used to measure water-soluble carbohydrates (WSC) calorimetrically (Arthur Thomas, 1977). The pH was evaluated with a glass electrode pH meter (pH221, Hanna Ltd., Italy). The methods of Barker & Summerson [9] and Chaney & Marbach [10] were used to determine the concentrations of lactic acid and NH_3-N, respectively. In accordance with Shao et al. (2005), volatile fatty acids (VFAs) were measured using gas chromatography (Shimadzu GC-17A, Sigma-Aldrich Co.) equipped with a flame ionization detector. The Playne & McDonald [11] approach was used to analyze the buffering capacity of fresh material.

2.3 Microbial Population Analysis

After macerating 10 g of silage samples in 90 mL of sterile water with a medium-speed blender, the samples were serially diluted. After being grown on de Man, Rogosa, and Sharpe (MRS) agar, LAB were incubated anaerobically for three days at 37°C. Aerobic bacteria and yeasts were counted on nutrient agar (NA) and potato dextrose agar (PDA), respectively (Shanghai Bioway Technology Co., Ltd.). Microbial counts were expressed as $\log_{10}$ cfu/g wet weight.

2.4 Statistics Analysis

SAS statistics software was used for all statistical analyses [12]. Statistical significance was established at $P<0.05$.

3. Results

Tables 1 and 2 and Figure 1 show the chemical makeup and fermentation characteristics of King Grass both before and after ensiling. All LAB-treated silages showed a substantial ($P<0.05$) rise in lactic acid (LA) and propionic acid (PA) and a significant ($P<0.05$) drop in pH, acetic acid (AA), water-soluble carbohydrates (WSC), and butyric acid (BA) when compared to the control. Figure 2 and Table 3 give an overview of King Grass silage's chemical and fermentation properties. Among the treatments, the commercial *Lactobacillus plantarum* strain demonstrated superior performance compared to the isolates from Italian Ryegrass and Napier Grass across all sampling periods (days 14, 30, and 60). Table 4 illustrates the microbial composition during ensiling, showing a significant ($P<0.05$) decline in aerobic bacteria and yeast populations, while LAB populations increased considerably.

Table 1. Chemical composition of king grass before ensiling

Items	Mean
DM (g/kg FM)	167.92
WSC (g/kg DM)	38.94
NDF (g/kg DM)	708.25
ADF (g/kg DM)	429.81
AB (Log_{10} cfu/g FM)	3.68
Yeast (Log_{10} cfu/g FM)	3.64
LAB (Log_{10} cfu/g FM)	4.03

DM, dry matter; FM, fresh matter; Log, Denary logarithm of the numbers of bacteria.

Table 1. Fermentation qualities of king grass with LAB strains during ensiling

Item	Ensiling day	KC	KP	KN	KI	Significance			
						Standard Error	T	D	T × D
pH value	14	3.76^{aA}	3.61^{bB}	3.57^{bB}	3.57^{bB}	0.020	<0.001	<0.001	<0.001
	30	3.64^{aA}	3.53^{bA}	3.43^{Cc}	3.46^{bBC}				
LA (g/kg DM)	14	61.68^{bB}	85.40^{aA}	85.68^{aA}	81.66^{abAB}	0.393	<0.001	0.002	0.091
	30	71.12^{bB}	91.54^{aA}	89.52^{aA}	89.16^{abAB}				
AA (g/kg DM)	14	7.78^{aA}	2.36^{abAB}	2.25^{bAB}	2.90^{bB}	0.726	<0.001	0.003	0.002
	30	6.91^{aA}	2.45^{bAB}	2.59^{bB}	3.42^{bB}				
PA (g/kg DM)	14	0.37^{bB}	1.39^{aB}	1.57^{aA}	1.63^{aA}	0.072	<0.001	0.002	<0.001
	30	0.43^{bB}	1.96^{aA}	0.70^{abAB}	1.90^{aA}				
BA (g/kg DM)	14	0.91^{aA}	0.39^{cC}	0.46^{bB}	0.61^{abAB}	0.040	0.029	0.003	0.154
	30	0.76^{aA}	0.51^{bB}	0.57^{abAB}	0.54^{bB}				

Significant ($P < 0.05$) differences across ensiling days in the same treatment are indicated by values with distinct lowercase letters, while significant differences between treatments within the same ensiling day are indicated by the capital. Group (KN): L. plantarum HDASK; Group (KC): control (no additive); Group (KP): L. plantarum (KP, commercial additives); Group (KI): L. paraplantarum KI at a rate of 1×106cfu/g FM

Figure 1. Fermentation qualities of King grass with LAB strains during 60 days of ensiling

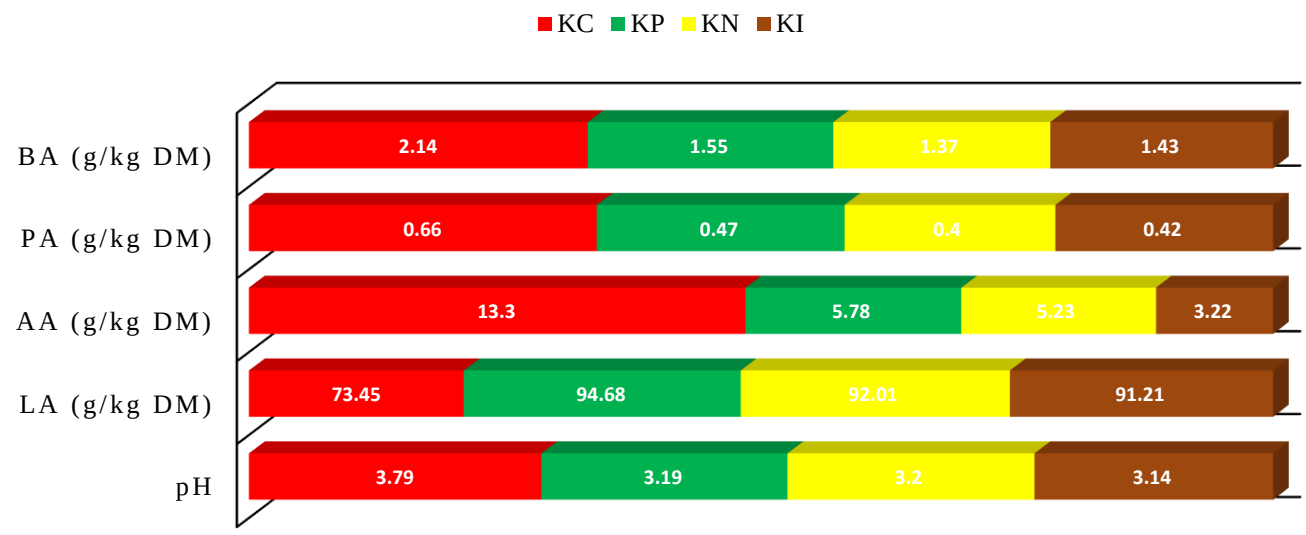


Table 2. Chemical compositions of king grass with LAB strains during ensiling

Item	Ensiling Day	KC	KP	KN	KI	Significance			
						Standard Error	T	D	T × D
DM (g/kg FM)	14	165.54^{aA}	167.91^{aA}	178.27^{aA}	158.55^{aA}	0.668	0.035	0.002	0.493
	30	158.21^{aA}	150.72^{aA}	159.46^{aA}	149.55^{aA}				
Ethanol (g/kg DM)	14	8.95^{abAB}	7.94^{bB}	8.99^{abAB}	11.3^{aA}	0.360	0.309	0.653	0.463
	30	10.86^{aA}	9.71^{aA}	7.95^{bAB}	9.78^{aA}				
NH_3-N (g/kg TN)	14	44.94^{aA}	30.31^{aAB}	25.85^{bB}	31.90^{aAB}	0.037	<0.001	0.003	0.422
	30	41.07^{aA}	35.08^{aA}	26.16^{bB}	34.88^{aA}				
WSC (g/kg DM)	14	10.50^{aA}	4.74^{aAB}	2.06^{cC}	2.29^{abBC}	0.676	0.154	0.002	<0.001
	30	9.11^{aA}	3.46^{aB}	2.35^{cC}	2.46^{bC}				

Significant ($P < 0.05$) differences across ensiling days in the same treatment are indicated by values with distinct lowercase letters, while significant differences between treatments within the same ensiling day are indicated by the capital. Group (KN): L. plantarum HDASK; Group (SKC): control (no additive); Group (KP): L. plantarum (KP, commercial additives); Group (KI): L. paraplantarum KI at a rate of 1×106cfu/g FM.

Figure 2. Chemical compositions of king grass with LAB strains during 60 days of ensiling

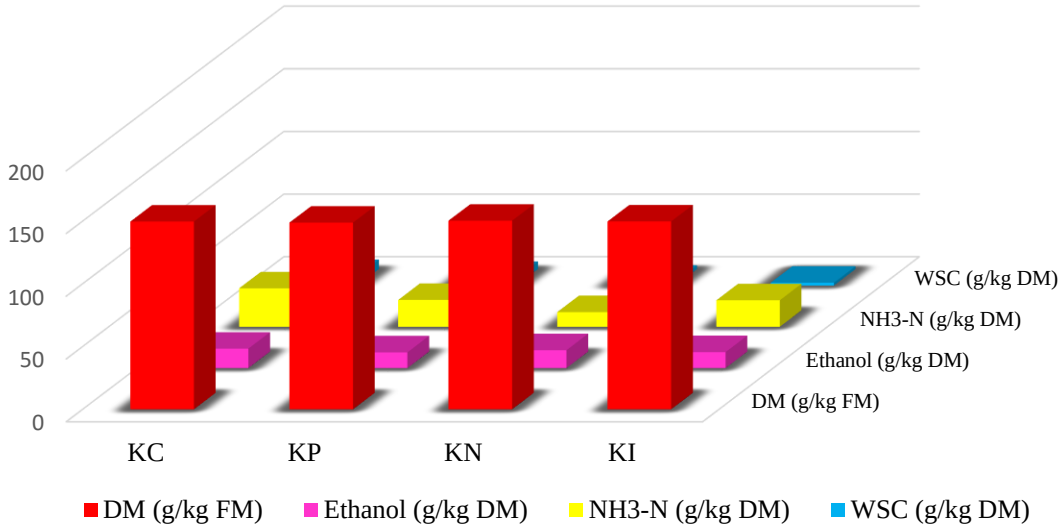
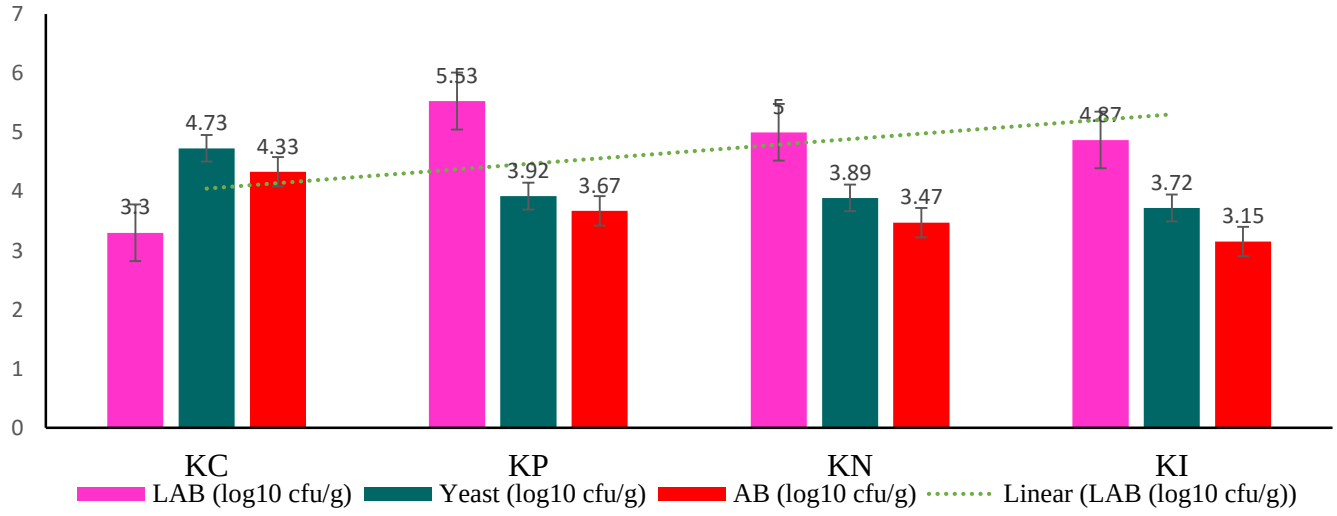


Table 3. Microbial compositions of king grass with LAB strains during ensiling

Item	Ensiling day	KC	KP	KN	KI	Significance			
						Standard Error	T	D	T × D
LAB (log_{10} cfu/g)	14	5.13^{bC}	5.20^{bB}	5.22^{bB}	5.26^{aA}	0.134	0.460	<0.001	0.023
	30	4.15^{cC}	5.28^{aA}	5.40^{aA}	5.00^{aB}				
Yeast (log_{10} cfu/g)	14	4.62^{aA}	4.07^{aA}	3.95^{aA}	4.15^{aA}	0.134	0.191	<0.001	0.120
	30	4.71^{aA}	4.76^{aA}	4.56^{aA}	5.03^{aA}				
AB (log_{10} cfu/g)	14	3.66^{aAB}	3.23^{aAB}	2.95^{bB}	3.05^{aAB}	0.069	0.214	<0.001	0.128
	30	3.62^{aA}	2.59^{bB}	3.47^{aAB}	2.90^{bA}				

Significant ($P < 0.05$) differences across ensiling days in the same treatment are indicated by values with distinct lowercase letters, while significant differences between treatments within the same ensiling day are indicated by the capital. Group (KN): L. plantarum HDASK; Group (SKC): control (no additive); Group (KP): L. plantarum (KP, commercial additives); Group (KI): L. paraplantarum KI at a rate of 1×106cfu/g FM.

Figure 3. Microbial compositions of king grass with LAB strains during 60 days of ensiling



4. Discussion

The effects of several lactic acid bacteria (LAB) inoculants on King grass silage during varied ensiling times were investigated in this study. Both the pH levels and the concentrations of butyric acid (BA) and acetic acid (AA) dropped significantly ($P<0.05$). In line with our results, According to Kim et al. [13], LAB inoculation significantly ($P<0.05$) reduced pH, acetic acid, and butyric acid but had no influence on the dry matter (DM) content of silage.

Lactobacillus plantarum is typically thought to be more efficient than heterofermentative LAB because of its ability to quickly reduce pH and ammonia nitrogen (NH3-N) content [14]. Similarly, Wang et al. [15] discovered that the entire mixed ration silage and whole-crop corn had the lowest pH and the highest lactic acid level.

While acetic acid, water-soluble carbohydrates (WSC), butyric acid, NH3-N, and pH levels decreased in silage treated with LAB, lactic acid concentrations increased over 7, 14, 28, and 56 days of ensiling [16]. Numerous studies have also reported improved silage fermentation after LAB inoculation in barley (*Hordeum vulgare*), pearl millet (*Pennisetum americanum*), elephant grass (*Pennisetum* purpureum), and king grass (*Pennisetum purpureophoides*). When homolactic acid bacteria were added, either by themselves or in conjunction with other strains, the pH quickly dropped and the number of lactic acid bacteria [17].

According to Zhang et al. [18], high ethanol buildup may cause Napier grass silage's fermentation quality to deteriorate in the early phases of ensiling. Over the course of the 30-day ensiling period, the ethanol content rose in the current study. According to Filya [19], silage infected with *L. plantarum* exhibited greater levels of LAB, yeast, and aerobic bacteria than the control.

Several studies have found that using LAB inoculants during prolonged ensiling did not appreciably change the silage's DM content [15]. Additionally, during the 100 days of ensiling, Kim et al. [13] and Amanullah et al. [14] found no significant ($P<0.05$) changes in DM, crude protein (CP), neutral detergent fiber (NDF), or acid detergent fiber (ADF). Our results are consistent with earlier research that treated King grass with acacia tannin and ELAB [2].

Plant proteases convert proteins into peptides and free amino acids during the ensiling process [4]. Furthermore, the conversion of amino acids into ammonia and other nitrogenous non-protein molecules is mostly the result of proteolytic processes [20]. Ineffective or secondary fermentation, in which amino acids undergo AA synthesis to form NH3, is indicated by the creation of acetic acid, butyric acid, and other fermentation byproducts [2].
Consistent with earlier studies, untreated silage has lower amounts of NDF and ADF [1,2]. One reason for the drop in NDF and ADF concentrations is the enzymatic breakdown of cellulose and hemicellulose in the plant cell wall during ensiling. LAB-treated silage demonstrated improved nutritional value and digestibility due to the lower fiber content [21].

5. Conclusions

King Grass silage's fermentation quality and stability were enhanced overall by LAB inoculation, with *L. plantarum* showing the most encouraging results.

Acknowledgments

The present work is supported by Nanjing Agricultural University's experimental grassland in China.

Financial support and sponsorship
The study was not received any internal or external funds.

Conflicts of interest
There are no conflicts of interest.

References

[1]. Yahaya, M., M. Goto, W. Yimiti, B. Smerjai and Y. Kawamoto (2004). Evaluation of fermentation quality of a tropical and temperate forage crops ensiled with additives of fermented juice of epiphytic lactic acid bacteria (fjlb). Cellulose, 31: 28.25.

[2]. Li, M., X. Zi, H. Zhou, G. Hou and Y. Cai (2014). Effects of sucrose, glucose, molasses and cellulase on fermentation quality and in vitro gas production of King grass silage. Animal Feed Science and Technology, 197: 206-212.

[3]. Santoso, B., B.T. Hariadia, H. Manik and H. Abubakar (2011). Silage quality of King grass (pennisetum purpureophoides) treated with epiphytic lactic acid bacteria and tannin of acacia. Media Peternakan-J. Anim. Sci. Tech. 34(2):140-145.

[4]. Abdelbasset, M. and K. Djamila (2008). Antimicrobial activity of autochthonous lactic acid bacteria isolated from algerian traditional fermented milk “raïb”. African J. Biotech. 7(16):2908-2914.

[5]. Ammor, S., G. Tauveron, E. Dufour and I. Chevallier (2006). Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility: 1—screening and characterization of the antibacterial compounds. Food control, 17(6):454-461.

[6]. Driehuis, F., S. Oude Elferink and P. Van Wikselaar (2001). Fermentation characteristics and aerobic stability of grass silage inoculated with lactobacillus buchneri, with or without homofermentative lactic acid bacteria. Grass and Forage Science, 56(4): 330-343.

[7]. Van Soest, P.v., J. Robertson and B. Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Science, 74(10): 3583-3597.

[8]. Arthur Thomas, T. (1977). An automated procedure for the determination of soluble carbohydrates in herbage. J. Sci. Food and Agri. 28(7): 639-642.

[9]. Barker, S.B. and W.H. Summerson (1941). The

colorimetric determination of lactic acid in biological material. J. Biological Chemistry, 138:535-554.
[10]. Chaney, A.L. and E.P. Marbach (1962). Modified reagents for determination of urea and ammonia. Clinical chemistry, 8(2): 130-132.
[11]. Playne, M. and P. McDonald (1966). The buffering constituents of herbage and of silage. J. Science Food Agri. 17(6): 264-268.
[12]. SAS (2003). Statistical analysis system user's guide; Statistics version 9.1; SAS Institute; Cary; NC; USA.
[13]. Kim, D.H., S.M. Amanullah, H.J. Lee, Y.H. Joo and S.C. Kim (2015). Effect of microbial and chemical combo additives on nutritive value and fermentation characteristic of whole crop barley silage. Asian-Australasian J. Anim. Sciences, 28(9): 1274.
[14]. Amanullah, S., D. Kim, H. Lee, Y. Joo, S. Kim and S. Kim (2014). Effects of microbial additives on chemical composition and fermentation characteristics of barley silage. Asian-Aust. J Anim. Sci., 27(4): 511.
[15]. Baah, J., W. Addah, E. Okine and T. McAllister (2011). Effects of homolactic bacterial inoculant alone or combined with an anionic surfactant on fermentation, aerobic stability and in situ ruminal degradability of barley silage. Asian- Austr. J. Anim. Sci., 24(3): 369-378.
[16]. Reich, L.J. and L. Kung (2010). Effects of combining lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage. Anim. Feed Sci. Tech.,159(3): 105-109.
[17]. Zhang, L., C. Yu, M. Shimojo and T. Shao (2011). Effect of different rates of ethanol additive on fermentation quality of napiergrass (pennisetum purpureum). Asian-Aust. J. Anim. Sci., 24(5):636-642.
[18]. Filya, I. (2003). The effect of lactobacillus buchneri and lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. J. DairyScience, 86(11): 3575-3581.
[19]. Santoso, B., B.T. Hariadia, H. Manik and H. Abubakar (2011). Silage quality of King grass (pennisetum purpureophoides) treated with epiphytic lactic acid bacteria and tannin of acacia. Media Peternakan-J. Anim. Sci. Tech. 34(2):140-145.
[20]. Adesogan, A.T., (2006). Factors affecting corn silage quality in hot and humid climates. In: 17th Florida Ruminant Nutrition Symposium. pp:108-127.
[21]. Zhang, J.G., H. Kawamoto and Y.M. CAI (2010). Relationships between the addition rates of cellulase or glucose and silage fermentation at different temperatures. Anim.Science J. 81(3):325-330.