T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

EFFECTS OF ENCAPSULATED UREA AND INOCULANT ADDITION ON CORN SILAGE QUALITY AND GAS PRODUCTION PARAMETERS

Prepared by
Maryama KHALIF MOHAMUD

Supervisor
Prof. Dr. Yusuf KONCA

M. Sc. Thesis

September 2023
KAYSERİ

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COMPLIANCE WITH SCIENTIFIC ETHICS

Iˈm Maryama Khalif MOHAMUD, hereby declare that this piece of work is the result of my research and that this work has never been submitted anywhere for any degree. All the sources of information used were obtained by academic ethics and have been duly acknowledged.

Name- Surname: Maryama KHALIF MOHAMUD

Signature:

This master's thesis was written upon the topic "**Effects of Encapsulated Urea and Inoculant Addition on Corn Silage Quality and Gas Production Parameters”** was prepared with accordance Erciyes University's graduate Thesis Writing Directive

Prepared by

Maryama KHALIF MOHAMUD

Supervised by

Prof. Dr. Yusuf KONCA

Head of Department

Assoc. Prof. Dr. Özhan ŞİMŞEK

Under The Supervision of Prof. Dr. Yusuf KONCA, this write up on the topic " **Effects of Encapsulated Urea and Inoculant Addition on Corn Silage Quality and Gas Production Parameters"** was prepared by Maryama KHALIF MOHAMUD and was accepted as master degree thesis Department of agricultural sciences Technologies; Graduate School of Natural and Applied Sciences, Erciyes University.

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Jury:

Supervisor : Prof. Dr. Yusuf KONCA .....

Member : Prof. Dr. Sabri YURTSEVEN .....

Member : Dr. Öğr. Üyesi Mahmut KALİBER .....

Approval:

The acceptance of this thesis was based on decision number..... of the Executive Board of the Graduate School dated.....

…/…/2023

Prof. Dr. Emel KIZILKAYA AYDOĞAN

Director of Institute

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ENKAPSÜLE ÜRE VE İNOKULANT İLAVESİNİN MISIR SİLAJI KALİTESİ VE GAZ ÜRETİM PARAMETRELERİ ÜZERİNE ETKİLERİ

Maryama KHALİF MOHAMUD

Erciyes Üniversitesi, Fen Bilimleri Enstitüsü
Yüksek Lisans Tezi, Eylül 2023
Danışman: Prof. Dr. Yusuf KONCA

ÖZET

Bu çalışma, enkapsüle edilmiş üre ve inokulant ilavesinin mısır silajlarında kimyasal kompozisyon ve silaj kalite özellikleri ile aerobik stabilite ve mikrobiyolojik özellikleri üzerine etkilerini incelemek amacıyla yapılmıştır. Mısır bitkisi hamur olum zamanında hasat edilmiş ve üre (Ü), üreaz enzimli üre ve polimer kaplı üre ve inokulant ilave edilen gruplar hazırlanmıştır. Bu amaçla iki deneme planlanmıştır. Birinci çalışmada, gruplar şu şekilde oluşturulmuştur: 1: Kontrol (üre ve inokulant ilave edilmeyen silaj), 2: Mısır silajına %1 granül Ü ilavesi, 3: %1 üreaz enzimli üre (NBPT) ilavesi (BitÜ), 3: polimer kaplanmış yavaş salınımlı üre %1 (PCRU) ilavesi yapılmıştır. İkinci çalışmada, 4: silaja % 0,05 inokulant (IN, (L*actobacillus buchneri1x10^{14}Cfu/kg, Lactobacillus plantarum 8x10^{12}Cfu/kg,* and E*nterococcus faecium 2x10^{12}Cfu/kg* and enzymes; xylanase (endo-1.3-beta-xylanase) 2500000 FXU-W/kg and alpha amylase (1,4-alpha-amylase) 2000000 FAU-F/kg), Silamix, Royal A.Ş. Kayseri, Türkiye) ilavesi, 5:silaja % 0,1 IN ilavesi ve 6: silaja % 0,2 IN ilavesi yapılmıştır. Ayrıca silaja üre ve inokulant kombinasyonları oluşturulmuştur. Birinci çalışmada, silaja üre formları katılması kontrol grubuna göre ham protein (HP) oranını önemli derecede artırmış, ADF oranını azaltmış ($P<0.01$), ancak pH, kuru madde (KM), ham yağ (HY), kül (HK) ve NDF değerlerini önemli olarak etkilememiştir. Üre formları ilavesi aerobik stabilite, pH, CO_2, maya, küf ve lactobasil oranını önemli derecede etkilemiştir ($P<0,01$). Mısır silajına üre ilavesi asetik, butirik ve propiyonik oranlarını önemli derecede etkilememiştir. Üre ilavesi ile silajlarda toplam gaz üretimi azalmış ancak PCRU grubunda kontrole göre metan gazı oranı artmıştır ($P<0,05$). İkinci çalışmada silajlara inokulant ilavesi pH, KM, HK, ADF ve NDF oranlarını önemli derecede etkilememiş ancak kontrole göre HP oranı artmıştır ($P<0.01$). İnokulant ilavesi aerobik stabilite % ve pH düzeyini önemli derecede etkilememiş ancak maya, küf, laktobasil ve CO_2 oranlarında farklılıklara neden olmuştur ($P<0,01$). Silaja inokulant ilavesi asetik, butirik ve propiyonik oranlarını ve toplam gaz üretimini (24 saat)

önemli derecede etkilememiş, ancak %0,2 inokulant ilavesi diğer gruplara göre metan gazı oranını artırmıştır (P<0.01). Ana deneme gruplarındaki benzeri farklılıklar kombinasyonlarda da belirlenmiştir. Sonuç olarak, mısır silajına üre katılması silaj protein oranını, aerobik ve laktobasil sayısını artırmış, küf oranını ve toplam gaz üretimini azaltarak silaj kalitesine olumlu etkide bulunmuştur. Silaja inokulant ilavesi protein oranını ve aerobik stabiliteyi artırmıştır.

Anahtar Kelimeler: Silaj, Üre formu, İnokulant, Sürdürülebilirlik, Gaz üretimi

EFFECTS OF ENCAPSULATED UREA AND INOCULANT ADDITION ON CORN SILAGE QUALITY AND GAS PRODUCTION PARAMETERS

Maryama KHALIF MOHAMUD

Erciyes University, Graduate School of Natural and Applied Science
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Supervisors: Prof. Dr. Yusuf KONCA

ABSTRACT

This study was carried out to investigate the effects of encapsulated urea and inoculant addition on chemical composition, silage quality properties, aerobic stability and microbiological properties of maize silages. Corn plant was harvested at the time of pulping and urea (U), urease inhibitors urea (NBPT) and bacterial inoculant added groups were prepared. For this purpose, two experiments were planned. In the first study, the groups were formed as follows: 1: Control (silage without urea and inoculant added), 2: addition of 1% granule U to corn silage, 3: addition of 1% NBPT urea for plant (PlU) produced for plant fertilization, 3: Addition 1% polymer coated slow release urea for rumen (PCRU) were added. In the second study, 4: 0.05% inoculant (IN, (L*actobacillus buchneri1x10^{14} Cfu/kg, Lactobacillus plantarum 8x10^{12} Cfu/kg,* and E*nterococcus faecium 2x10^{1} ^{2}Cfu/kg* and enzymes; xylanase (endo-1.3-beta-xylanase) 2500000 FXU-W/kg and alpha amylase (1,4-alpha-amylase) 2000000 FAU-F/kg), Silamix, Royal A.Ş. Kayseri, Turkey) was added to the silage, 5: 0.1% IN was added to the silage, and 6: 0.2% IN was added to the silage. In addition, urea and inoculant combinations were created for silage. In the first study, the addition of urea forms to silage significantly increased the crude protein (CP) ratio and decreased the ADF ratio ($P<0.01$) compared to the control group, but pH, dry matter (DM), crude fat (CF), ash (CA) and NDF did not significantly affect the values. Addition of urea forms significantly affected aerobic stability, pH, CO_2, yeast, mold and lactobacilli rate ($P<0.01$). Addition of urea to corn silage did not significantly affect acetic, butyric and propionic ratios. With the addition of urea, the total gas production in silages decreased, but the methane gas ratio increased in the PCRU group compared to the control ($P<0.05$). In the second study, the addition of inoculant to silages did not significantly affect the pH, DM, CA, ADF and NDF ratios, but the CP ratio increased compared to the control ($P<0.01$). Addition of inoculant did not significantly affect the aerobic stability % and pH level, but caused differences in yeast,

mold, lactobacilli and CO_2 ratios ($P<0.01$). Addition of inoculant to the silage did not significantly affect acetic, butyric and propionic ratios and total gas production (24 hours), but the addition of 0.2% inoculant increased the methane gas ratio compared to other groups ($P<0.01$). Similar differences in the main experimental groups were also detected in the combinations. As a result, the addition of urea to corn silage increased the silage protein content, aerobic and lactobacilli count, decreased the mold rate and total gas production, and had a positive effect on the silage quality. The addition of inoculant to the silage increased the protein content and aerobic stability.

Keywords: Silage, Urea forms, Inoculant, Sustainability, Gas production

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ABBREVIATIONS

ADF	Acid detergent fibre
AS	Aerobic stability
CA	Crude ash
CF	Crude fibre
CO_2	Carbondioxide
CP	Crude protein
DM	Dry matter
PCRU	Polymer coated urea for ruminants
GP	Gas production
IN	Inoculant
Lb	Lactobacillus buchneri
ME	Mettabolizable Energy
N	Nitrogen
NDF	Neutral detergent fibre
NEL	Net energy lactation
OAs	Organic acids
OMD	Organic matter digestibility
P	Probability
NBPT	urease inhibitors urea (N-(n-tertbutyl) thiophosphoric triamide
U	Urea
WSC	Water soluble carbohydrate
ERUTAM	Erciyes University Agricultural Research and Application Center
AOAC	Association of Official Analytical Chemists
CH_4	Methane
ME	Metabolisable energy
NEL	Net energy lactation
OMD	Organic matter digestibility
DMD	Dry matter digestibility

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INTRODUCTION

Silage is produced by storing chopped whole plant and grain in an airtight silo. The feed storage method, which is based on the principle of preserving the green and water-rich feeds in an airless environment with the help of lactic acid bacteria, is called silage, and the feeds obtained by this method are called silo feeds. Silage, with its high water content roughage feature that minimizes the loss of value in nutrients, is used intensively in countries with advanced agriculture. The quality of corn silage is determined by its energy content and digestibility potential. Corn silage remains the most common feed fed to lactating dairy farms and it is becoming increasingly popular in the world. Because of its high digestible energy content, corn silage is an excellent feed for lactating cows. However, compared when compared to other high-quality feed feeds, corn silage contains significantly less rumen degradable protein (Boucher et al., 2007). Corn is one of the most important cereals grown and consumed worldwide. It plays an important socioeconomic role because of its many uses in human and animal nutrition (Da Costa Silva et al., 2020). However, the quality of the material to be silage and some features must be present in order to provide the expected benefit from silage (Konca et al., 2005). While a significant part of the silage produced is used in the feeding of dairy cows.

Since the protein content of the corn plant is low, it is far from to meet the protein requirements of ruminant animals. Therefore, it is necessary to supplement the feed of animals with protein-rich pulp to meet the required protein (Kutlu, 2002). Microorganisms in the rumen of ruminant animals such as cattle and sheep either synthesize protein from the feed they eat or convert protein using nitrogen (N) in non-protein nitrogenous compounds. Urea is a good source of N (46%) and used in animal feeds to complete N source for microbial protein synthesis in ruminants (Kutlu et al., 2005). Excessive and rapid fermentation of urea in the rumen can cause toxic for animals. It is a common practice to add urea as additive to corn silages during the ensiling process. Since corn silage usually typically contains an excess of easily readily fermentable

carbohydrates, cattle fed in this manner can readily utilize the urea nitrogen in the urea (Clark et al., 1973).

Protein feed sources are more expensive than grains and are utilized less often. Urea addition to diets may help to provide enough N content in feeds, so adding of urea to silage is a common and cheap way to increase the amount of nitrogen (N) available to ruminants. Urea infusion increased the silage's total nitrogen content, but quality of the fermentation was compromised due to the rise in pH caused by the dropping of ammonia (Makkar et al., 2007). However, there are some rules to use urea in animal diets, and they should be limited 1-1.5% and shouldn't give non ruminants and un-developed rumen animals like calves and lambs. To enrichment of diets N or protein concentration, urea addition may be used for this purpose (Panday, 2010).

Urea addition may improve silage quality via increasing dry matter, PH, and crude protein while decreasing ADF, NDF, acetic, butyric, propionic, and lactic acids in silages, also, it decrease of undesirable yeast in silages (Demirel et al., 2003).

In another way to improve the fermentation quality of silages, various inoculants, especially lactic acid bacteria (LAB) have been added and changed some properties of silages (Baytok et al., 2005). In addition, some new products are in developing such as microbial-derived enzymes and inoculant enzyme combinations, acids and regulators are in used the silo (Reich and Kung, 2010).

However, when urea, one of the silage additives, is added to the silage, providing a good distribution and loss from the environment due to the volatility of N may be in question. Thanks to the technological advances developed in recent years, there are important publications that the slow dissolution of urea in both plant nutrition and animal nutrition increases the usefulness (Ceconi et al., 2015; Granja-Salcedo et al., 2019; Lira-Casas et al., 2019).

Therefore, in this study, the effects of urea type (regular and encapsulated urea) and inoculant usage on corn silage quality characteristics, methane gas production and digestibility were examined.

CHAPTER 1

GENERAL INFORMATION AND LITERATURE REVIEW

1.1 Silage production

Corn silage is a very popular feed for ruminants in the world. Because of its ease of use and good digestion. Corn silage is an excellent source of feed for feedlots and lactation cows. Corn silage, on the other hand, contains substantially less protein (Boucher et al., 2007).

In order for ruminant animals to be fed and their digestive systems to work well, a certain amount of roughage must be included in the ration mixtures. The roughage used in the feeding of ruminant animals includes legumes and grasses as well as some agricultural by-products. In recent years, one of the most widely used roughage in intensive ruminant animal production is corn silage. With the use of corn silage, intensive ruminant animal feeding possibilities have also increased. Corn can be grown easily in countries with irrigated agriculture or in sufficient annual rainfall. As maize is produced for human nutrition, silage maize is planted in very large lands in order to make maize silage, especially for use in animal feeding. When the harvest time comes, depending on the size of the farm, tons of corn silage can be produced and used as the main roughage source throughout the year. Corn is one of the most widely used forage crops because it is easy to grow and reach, contains enough easily soluble carbohydrates for fermentation, is easy to digest, and is a source of good quality roughage (Kutlu, 2002). In addition, it is among the important advantages of corn plant to be planted and harvested twice a year in regions with hot climates and to obtain high yields due to the improved varieties.

Beneficial bacteria growth may potentially have an impact on silage quality. Silo conditions promote the proliferation of streptococcus, molds, yeast and other beach

bacteria, all of which lower the pH (Drouin et al., 2016). Lactic acid bacteria are helpful microorganisms that must be present in appropriate quantities in silos. Other bacteria that make butyric and acetic acid should be reduced in number and secretion (Elferink et al., 2000). Additives are applied to ensiled silage to prevent or minimize the growth of unwanted microorganisms in silages, hence improving silage fermentation and aerobic fermentation (Queiroz et al., 2013).

To increase silage quality specific lactic acid bacteria (LAB) strains have been developed. The main function of homofermentative inoculants is to provide rapid and efficient fermentation of water-soluble carbohydrates (WSC) to lactic acid, rapid pH reduction, and improved silage preservation with minimal fermentation losses (Okoye et al., 2023).

The maturation of silages and their desired properties are the result of the fermentation of beneficial bacteria. To obtain a good fermentation and increase value of silage quality some additives have been applying such as grains, molasses, inoculants, enzymes, acids, urea. These additives are typically used to increase fermentation efficiency and aerobic stability while reducing sanitary issues (Sucu and Filya, 2006). *Lactobacillus buchneri* is a heterofermentative LAB and have been using to inhibit yeast and mold growth in silos (Holzer et al., 2003). Studies have demonstrated that using inoculant, urea, and inoculant increases silage quality while decreasing aerobic stability (Nsereko et al., 2008; van Gastelen et al., 2015 ; Araki et al., 2017; Vieira et al., 2017). Since urea is a potent antifungal, it raises the pH of silages and inhibits yeast growth. It can support the growth of these microorganisms, which encourage heterofermentative LAB to create acetic acid in order to maintain the silage's pH in an appropriate range (Neumann et al., 2010)

Silage inoculants are used to enhance preservative performance and animal performance. Most commercially available inoculants contain homofermentative lactic acid bacteria (LAB), which create lactic acid quickly and effectively, promoting natural silage fermentation (Weinberg and Muck, 1996).

1.2. Effect of urea addition on silage quality

It is now common practice to add urea to corn silage during the ensiling process. Since corn silage usually typically contains an excess of easily readily fermentable

carbohydrates, cattle fed in this manner can readily utilize the urea nitrogen in the urea. However, some urea nitrogen losses are common.

It is a well-known practice supplementation of urea to corn silage at a time of ensiling. Since corn silage usually typically contains an excess of easily readily fermentable carbohydrates, cattle fed in this manner can readily utilize the urea nitrogen in the urea. However, some urea nitrogen losses are common. These losses can be exacerbated when corn silage has low energy content due to drought conditions that allow little or no air formation (Clark et al., 1973).

Supplementation to corn silage 0.75% and 0.5% urea when compared to non additive (control) group, pH increased, and acetic acid to 0.75% while decreasing to 0.5%, and propionic, butyric, and lactic increase with different percentages (Shirley et al., 1972).

Sariçiçek and Kiliç (2009) found that the fermentation kinetics, gas production, and energy content of corn silage quality, when 1% urea was added to the corn silages. Test silages were wilted for 6 hours, preserved in jars, and analyzed in 60 days. Corn silage samples showed that the pH and metabolisable energy (ME) were become non-significant, although the levels of dry matter and net energy (NE) were significantly decreased, acetic, butyric, and lactic acids were also showed non significance. In the process of gas production, for the first six hours decreased while 12 and 24 hours were looked non significance.

Corn silage were added 0.5% of urea to determine the quality parameters of corn silage, So the addition of 0.5% urea to corn silage resulted in an increase in dry matter, PH, and crude protein while conversely resulting non significance in NDF, ADF Acetic, and Butyric while Propionic has significance decreased (Demirel et al., 2003).

Sánchez-Meraz et al. (2014) found that the addition of slow-release urea did not affect gas production and degradation of alfalfa hay and oat straw. Dry matter and neutral detergent fiber in corn silage were non significance. Slow release urea addition to stover and corn silage caused an increase in *in-vitro* degradation and gas production.

On the other hand, the corn silage quality strictly bound its harvest time, fertilization, corn cultivar, climate, and fullness of corn stalk (Sheaffer et al., 2006). Corn silage must be collected when the moisture content is appropriate for feed use.

Making silage from different water content higher plants (more than 60%) to preserve long time and use for feed for ruminants is common practice. The goals of ensiling are to keep crop health quality throughout storage with low DM and energy waste. Plant respiration and proteolytic action, the clostridia fermentation process, and aerobic microbial activity are all major hurdles in the creation of high-quality silage (Muck, 1988).

Lizarazo et al. (2014) determined the impact of corn silage with the addition of urea and slow-release urea (SRU) on corn silage quality, after ensiling result showed that decrease in DM, NDF, and a slight increase in acetate and propionate, and a decrease in butyrate. Also decreased ruminal pH and reduced rumen ammonia, but did not improve total tract digestibility or microbial protein synthesis.

Kang et al. (2018) analyzed the nutritional content of cassava top silage with different dose of urea 0.5 and 1% urea, as well as gas generation. The silage pH, DM, organic matter (OM), and crude protein (CP) all increased. ADF % was declined however NDF raised by 0.5% while decreasing by 1% urea addition.

Weinberg et al. (2013) identified the quality of corn silage stored for varying amounts of time up to a maximum of one year by examining samples taken after one week, two weeks, three weeks, one month, three months, six months, and 12 months. The examination discovered quickly dropped pH of the corn silage, and after only one week, it dropped below the 4.0 level. The pH levels rose to 4.1–4.2 after being stored for three months. Corn silage's dry matter (DM) content and NDF digestibility dropped during a year of storage. The corn silage's lactic acid amount reached a maximum one month after being ensiled and then began to decline, while its acetic acid content increased. After three months of storage, the amount of dry matter losses reached its maximum. During the aerobic stability test, the storage period’s influence was significant for the DM content, NDFD, DM losses, acetic acid generation, and CO2 production.

Addition to rice silage urea (U0, U0.5, U1.0, U1.5, and U2.0%) caused and increase pH of the silages with increasing of U ratio in silage. The addition of molasses or U had no effect on the dry matter, acid detergent fiber (ADF) and organic matter (OM) of the silages. TVFA (acetic acid, and propionic acid) decreased (Wanapat et al., 2013).

On silage quality and in vitro digestibility of 0.5% urea or 0.5% urea plus 4% molasses addition to silage caused an increase the pH and crude protein (CP) of the silage. While silage acetic acid levels were unaffected by urea and molasses addition and silage DM digestibility was enhanced. Silage's lactic acid level was lowered by the addition of urea or urea with molasses (Nursoy, 2003).

The addition of 1.5% urea, 10% crushed corn grain, and 0.5% mineral premix to sugarcane silage showed a negative linear relationship between fermentation duration and true protein levels, but crude protein (CP) and lactic acid (LA) levels exhibited a positive linear trend. The time of fermentation exhibited a quadratic relationship with the pH of the silage. Furthermore, the process of micro-silage ensilaging involving the use of whole-stalk corn ensiled with stem sugarcane enhanced with corn grain, urea, and minerals (referred to as SSCE) was examined. The results indicated that the chemical composition of the silage remained unchanged after a period of 30 days (Gómez-Vázquez et al., 2011).

An experiment was conducted to evaluate the efficacy of incorporating urea as a supplement in silage production using different combinations of melon biomass. The composition of the silage samples exhibited different proportions of fruit, ranging from 0% to 100%. The incorporation of urea in the silage constituted the secondary variable in the subsequent manner: The silage with the addition of urea exhibited the highest crude protein content, measuring 69.8 g/kg DM. This particular silage variant comprised urea levels ranging from 0% to 1.5%. The silages containing 0% and 100% fruit, with respective fruit concentrations of 0.6% and 1.13%, exhibited the most significant gas (GAS) loss when urea was not included (Nascimento et al., 2023).

Urea was added to alfalfa silage at concentrations of 0.5% and 1%. After a period of 15 days, the silage treated with 1% urea exhibited elevated pH levels, a greater ratio of ammonia-N to total nitrogen (NH3-N/TN), and a larger content of crude protein (CP) compared to both the control group and the silage treated with 0.5% urea. Conversely,

the silage treated with 0.5% urea shown a higher content of acid detergent fiber (ADF) in comparison to the silage treated with 1% urea (Hou et al., 2022).

Sánchez-Meraz et al. (2014) found that the addition of slow-release urea affected gas production (GP) and degradation of DM and NDF in corn silage and GP were significantly increased.

1.3. Effect of bacterial inoculants on silage quality

The addition of a LAB inoculant to the corn silage quality parameters increased the PH, Crude protein, organic matter, and Acetic acid. Although the NDF, WSC, and Lactic acid levels fell, the dry matter remained unchanged (Meeske and Basson, 1998).

Queiroz et al. (2012) investigated the chemical composition of corn silage to determine the impact of using an inoculant bacteria. Dry matter, crude protein, natural detergent fiber (NDF), and water-soluble carbohydrates increased, while acid detergent fiber (ADF) and ash decreased.

Stokes et al. (1994) reported that inoculant treatment did not affect silage pH, however, it increased acetic acid concentration, titratable acidity, and DM loss during fermentation. The cellulose, NDF, ADF, and hemicellulose concentrations in the silage were decreased by 11 to 13% ratio, and WSC levels were 20% lower than in the control silage.

Addah et al. (2011) found that the addition of bacterial inoculant to the corn silage resulted increase of pH, ADF, NDF, WSC, acetate, lactate, and propionate. While Dry matter, crude protein, and yeast were decreased.

Rust et al. (2013) when LAB inoculated to silage had a lower temperature and higher lactic acid levels than control. The addition of microbial inoculant to the corn silage had no effect on its nutritional value, but it appeared to reduce the corn silage's stability to air exposure.

Yuan et al. (2015) found an increase in the PH value and Organic acids such as lactic acid, acetic acid, propionic acid, butyric acid, VFAs, Lactic acid bacteria, aerobic bacteria, and yeast after treating the silage with Lactobacillus plantarum. Furthermore, the dry matter, crude protein, and ash content of inoculated silages are increased, Due to

the decreased degradability of dry matter and NDF, in vitro gas production increased from 34.5 to 36.8.

Khorvash et al. (2006) investigated whether inoculants and absorbent material enhance the quality of corn silage. They compared corn silage with various dry matter levels (29% and 20%) and added commercial inoculants containing *Lactobacillus plantarum and Propionic bacteria sp.* to both corn silage samples. In addition, 0.5% zeolite and 0.5% limestone, 1% bentonite, 1% zeolite, 5, 15% or 10, pulverized barley, 5, 10, or 15% powdered whey, and 10, 5, or 15% dehydrated molasses were added. Consequently, silages indicate an increase in pH, acetic and lactic acids, dry matter, and improvement in vitro dry matter degradability. The addition of whey, or barley Feedtech inoculant improved in vitro DM degradability of corn silage.

Bağcık et al. (2022) determined that addition of urea have no significance on silage pH, DM, propionic, acetic, butyric acid ratios. Preactivated *Lentilactobacillus buchneri* in combination with urea improved nutritional quality, fermentation characteristics, and aerobic stability in corn silages while lowering methane levels.

In a another study, Moselhy et al. (2015) investigated the effect of LAB and urea on the chemical composition, microbial populations, and in vitro digestibility of silage and they found that the pH, lactic, butyric, propionic acids, crude protein, crude ash, ADF, and NDF, and aerobic stability are increased while acetic was decreased. Silages had lower levels of enterobacteria, clostridia, and yeast, no molds, and greater quantities of LAB.

Arriola et al. (2011) found that corn silage supplemented with a mixture of *Pediococcus pentosaceus* and *Lactobacillus buchneri*, 0.1% of Silage Savour acid mixture, 0.1% of sodium benzoate, *Lactobacillus plantarum, Acetobacter pasteurianus-ATCC 621, and Acetobacter pasteurianus-ATCC 9323,* and it was ensiled for 120 days. All parameters are non-significant to the DM% after three days of fermentation, but pH, lactic acid, and acetic acid increase their significance. After 120 days, fermentation and yeast parameters are observed; DM%, lactic acid, and yeast levels are not significant, whereas PH, acetate, and butyrate levels increase significantly.

Queiroz et al. (2012) determined that adding *5×105cfu/g of Lactobacillus buchneri and Pediococcus pentosaceus* bacterial inoculant to corn silage decreased the quantity and

percentage of spoiled silage by over 50%. As a consequence, the pH of inoculated silage has decreased; crude protein, gross energy, ash, ADF, and NDF have also decreased, while it inhibited yeast growth.

Stokes et al. (1994) examined a mixture of bacterial inoculants containing 80 to 90 percent *Pediococcus acidilactici* and 10 to 20 percent *Lactobacillus plantarum,* and their effects on the fermentation quality of corn silage and analyzed on different days until 56 days. The results indicate that until 7 days of fermentation, ADF, NDF, and pH decrease, although days 2 and 7 did not affect silage pH. After the final day of fermentation, the pH, ADF, NDF, hemicellulose, cellulose, and water-soluble carbohydrate concentrations were lowered.

Addah et al. (2011) discovered that adding bacterial inoculants *Lactobacillus plantarum, Pediococcus acidilactici,* and *Enterococcus faecium* to corn silage improved the aerobic stability, fermentation characteristics, and nutritive value of corn silage by increasing the pH, NDF, ADF, WSC, lactate, acetate, and propionate. Dry matter, crude protein, and yeast are all decreased.

Contreras-Govea et al. (2011) found that different bacteria were added to corn silage using different methods. The first group applied *lactobacillus plantarum* and *E. faecium* (*LP-EF*), the second group added lactobacillus plantarum only (LP), the third group applied *Lactobacillus pentosus* (*LPe*), and the fourth group added *lactobacillus lactis* (*LL*). As a result, the lactic acids LP-EF and LPe decrease while LP and LL increase, and the pH of all the different inoculants rises. Acetic acid ratios increased in all groups except LL, also the ethanol of all groups increase except LPe. Dry matter increases in Lp-EP and LL and control was both are same, while LP and LPe were decreased. ADF level increased all except LP.

Meeske et al. (1998) reported that the addition of lactic acid bacteria to the corn silage to investigate nutritional quality parameters of the corn silage, so result showed that the increase of pH, crude protein, organic matter, and acetic acid. Yeast and mold count increased and NDF, WSC, and lactic acids levels decreased, dry matter levels remained without change.

Yuan et al. (2015) found an increase in the pH value and organic acids such as lactic acid, propionic acid, acetic acid, butyric acid, VFAs, LAB, aerobic bacteria, and yeast after treating the silage with *Lactobacillus plantarum*. Furthermore, the dry matter, crude protein, and ash content of inoculated silages are increased, Due to the decreased degradability of dry matter and NDF, *in vitro* gas production increased from 34.5 to 36.8.

Acosta Aragón et al. (2012) identified corn silage treated with a commercial inoculant to describe the nutrient content, fermentation, and aerobic stability of the silages. When the inoculated silages the pH, total organic acids, lactic, and acetic acids all significantly increased, while butyric acid, ethanol, and ammonia significantly decreased compared to control group.

Aksu et al. (2004) discovered that the addition of inoculant to the corn silage to determine the characteristics of corn silage fermentation. As a result of pH, ADF, NDF, Dry matter, butyric acids, and organic matter were reduced, while crude ash, crude protein, lactic acid, and acetic acid were increased.

Sharp et al. (1994) observed that addition of two LAB inoculants (*Lactobacillus plantarum* and *Streptococcus fecal*) to grass silages did not effect on the DM, organic matter, NDF, ADF, ammonium nitrogen, and ethanol are non-significant. However, it changed the pH, acetic, propionic, and butyric acids and have significantly decreased their ratio in silages.

Baytok et al. (2005) claimed that inoculant supplementation into the corn silage caused a significant increase in pH, ash, and crude protein, however, ADF, NDF, and Dry matter had significant decrease, also the acetic, propionic, and butyric acids increase.

da Silva Ávila et al. (2010) determined silage quality parameters, to define the effect of microbial inoculants containing *L. buchneri or L. Plantarum* on sugar cane silage. Thus, the result of ADF, NDF, Dry matter, yeast, and propionic acid decreased, whereas the crude protein, LAB, lactic acid, and acetic acid increased.

Kamarloiy and Yansari (2008) discovered that the addition of Lactobacillus plantarum, and Propionibacterium acidipropionici to corn silage nutritional quality parameters, so, the result indicates an increase silage dry matter content, NDF, crude protein, and ash.

In a study, the addition of corn silage with homofermentative and heterofermentative inoculants were analyzed the DM, CP, OM, and ash contents and the concentration of NH_3-N increased with time after ensiling, while the remaining WSC and CP contents decreased. The concentration of NH3-N increased with time after ensiling, while the remaining WSC and CP contents decreased. The pH of corn silage inoculated with the homofermentative inoculant was the lowest after only 28 days. The inoculation of corn silage had a significant impact on ruminal DM degradability but did not affect ruminal CP degradability. Inoculation received a significant impact on the amount of gas produced by corn silage (Bayatkouhsar et al., 2012).

Cleale et al. (1990) reported that addition of *Lactobacillus xylosus* and *Pediococcus acidilactici* inoculants in corn silage increased the dry matter % and WSC of inoculated silage compared to control silage. Inoculated silage had lower pH, ADF, NDF, mold and yeast counts, also levels of all organic acids such as propionic, butyric, and acetic acids are lower than control silage.

Nair et al. (2020) investigated the effects of *heterofermentative* lactic acid bacteria (*Lactobacillus hilgardii* and *Lactobacillus buchneri*) inoculation on corn silage and fermentation properties. The pH of the silages treated with *Lactobacillus buchneri* was higher, VFA increased when inoculant was added, and inoculated silages had higher ADF and NDF contents than control silages. *Lactobacillus buchneri* had lower yeast and mold counts. Total bacterial and LAB populations did not differ between treatments.

Jin et al. (2015) noted that all inoculated corn silages had less water-soluble carbohydrate and more DM loss than the Control; inoculant supplementation to silages also had a higher pH and produced more acetic acid and ethanol but less lactic acid than the control. After a 21-day exposure to oxygen, all inoculated silages remained stable, whereas the control silage degraded, as indicated by a decline in lactic acid, an increase in pH, and yeast counts.

To promote fermentation and preservation or to inhibit harmful detrimental processes in a silo, chemical additives such as urea and CaO are available. *Lactic acid bacteria* thrive in the presence of additives. Silage additives are mixed in during storage (Yunus et al., 2000).

The parameters utilized to establish the index for silage quality were the digestible neutral detergent fiber (NDF) content after a 30-hour in vitro incubation (expressed in grams per kilogram of NDF) and the dry matter (DM) concentrations. The fundamental components considered in this study include starch, crude protein, ether extract, ammonia, and lactic acid (Tharangani et al., 2021).

Zahiroddini et al. (2004) defined corn silage preserved with inoculant and inoculant with enzyme (cellulose and amylase) to assess the qualitative features of the silage. Both DM and crude protein levels increased as a result of the experiment, pH and organic matter decreased. NDF increased the inoculant+enzyme group whereas ADF increased the inoculant group. Lactic acid and butyric acid levels are also high. Acetic acid decreased whereas propionic acid increased in the inoculant group.

Zahiroddini et al. (2006) evaluated different types of inoculants used with corn silage to better understand the characteristics of aerobic stability, nutrient retention, and fermentation. As a result shown that the NDF increased while crude protein decreased, and pH and dry matter increased. Acetic acid production by *L. plantarum*, *Enterococcus faecium*, *Lactobacillus buchneri*, and hydrolytic enzymes increases, but lactic acid and propionic acid production decreases. As well as mold decreased, but yeasts increased.

Sucu et al. (2006) discussed how lactic acid bacteria inoculants impact the aerobic stability, fermentation, and rumen degradability of wheat silages. As a result, inoculants increased *Lactobacillus* levels while decreasing yeast and mold levels in silages. The inoculant treatment increased the aerobic stability of the silages, but had no influence on the degradability of the wheat silages.

In order to separate the quality characteristics of corn silage, corn silage was evaluated after being mixed with two additives: microbial inoculant and cellulase. Both of the additions cause a reduction in pH while increasing DM levels. Additionally, ADF and NDF fall to lower levels (Kung et al. ,1990).

Filya et al. (2007) evaluated some of microbial inoculants to know the effect of nutritive value and fermentation on alfalfa silage was studied under laboratory conditions. (Pioneer 11A44*)*, *L. buchneri* (Biotal), *faecium* (Pioneer 1174), (Biomate LP/PC), *L. plantarum* (Biomax5), *Propionibacterium jensenii* and *Pediococcus pentosaceus* (Biotal Plus), *L.*

plantarum, E. faecium, and Pediococcus spp. (H/M Plus), the DM and pH of inoculated silage decreased ,Although the lactate, acetate, NDF and ADF increased.

Filya et al. (2000) found that wheat silage with two inoculants, a combination of *Enterococcus faecium*, *Lactobacillus plantarum* and *Lactobacillus pentosus*, had a low pH and a higher mold count. The silages inoculated with inoculant (*L. pentosus*) were more stable than the other group, but yeast was not found, while lactic acid and acetic acid increased.

Filya et al. (2006) evaluated the impact of P. acidipropionici, L. plantarum, and a combination of P. acidipropionici and L. plantarum on the fermentation process and aerobic stability of corn silage. The independent application of P. acidipropionici and L. plantarum did not result in an enhancement in the aerobic stability of low dry matter (DM) silages. Furthermore, silages infected with L. plantarum exhibited significantly elevated levels of lactic acid compared to the control group. The levels of lactic acid and pH in corn silage exhibited a decrease in comparison to the control group, whereas the levels of acetic, propionic, and butyric acid were unchanged.

Filya (2003) has shown the impact of *Lactobacillus buchneri*, alone or in combination with *Lactobacillus plantarum*, on the fermentation and aerobic stability of low dry matter corn silage. Silage lactic acid and acetic acid levels increased, and pH dropped while DM remained the same. Yeast and mold counts have both been reduced.

Luther (1986) investigated the effect of two different dry matter contents—39 and 30% DM% on nutrient preservation while utilizing a commercial inoculant containing *Lactobacillus plantarum*. There were two experiments planned for experiments 1 (39%DM) and 2 (30% DM). In experiment 1, butyric acid decreased whereas acetic acid stayed constant for the control and both lactic and propionic acids increased. In experiment 2, the amounts of lactic, propionic, and butyric acids decreased while dry matter and pH increased.

A commercial silage inoculant containing Lactobacillus plantarum, on corn silage that was treated *with Lactobacillus plantarum* and *Bacillus subtilis* and either supplemented or not supplemented with *amylase*, acetic, butyric, and pH levels all dropped but propionic levels increased. however DM, OM, crude protein, Yeast and mold counts are

all dropped, NDF levels increased (Lara, 2017). Similar results were found by (Danner et al., 2003).

Weinberg et al. (2002) found that the levels of lactic acid, acetic acid, and pH in corn silage that had been treated with *lactobacillus buchneri* alone or in combination with *lactobacillus plantarum* were significantly decreased. The number of yeast and mold colonies decreased at the same time.

Weinberg et al. (1995) found that sorghum silage treated with either LAB inoculant (*Lactobacillus plantarum* or *Pediococcus cerevisiae*) or *Propionibacterium shermanii* (PAB) grew better than sorghum silage treated with only LAB inoculant (*Lactobacillus plantarum* or *Pediococcus cerevisiae*). The control silage and the silage that had PAB added to it were constant but the silage that had LAB added to it increased.

Weinberg et al. (1995) evaluated two distinct inoculants, *Propionibacterium shermanii (PS),* alone or in combination with lactic acid bacteria inoculants (LAB) in corn silage. The pH, crude ash, crude protein, and ADF levels were all significantly decreased. Mold levels increased but yeast counts decreased. In another experiment Weinberg et al. (1993) evaluated the use of corn silage with three different inoculants, two of which included *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus acidilactici*, and one of which had just *Enterococcus faecium*. As a result, the pH and crude ash decreased but the crude protein increased.

Koc et al. (2008) examined the biological composition of corn silage collected at the milk stage and the addition of *Lactobacillus plantarum*, *Pediococcus acidilactici*, and amylase. Lactic acid and DM levels increased whereas acetic acid, pH, and crude protein levels declined. Yeast and mold levels also increased.

Kizılsimsek et al. (2007) investigated the addition of lactic acid bacteria *Lactobacillus lactis, L. plantarum* Aber F1, and *L. plantarum* L-54 to alfalfa silage inoculant. Microbial populations (fresh matter basis), DM, and chemical composition (DM basis) of chopped alfalfa silages after 60 days of ensiling found that DM, Lactic acid, ADF, NDF, acetic acid, and pH increased while ADF, NDF, acetic acid, and pH declined.

Hristov et al. (2002) examined the effects of incorporating corn silage with different DM contents, specifically 30.7% DM or 37.8% DM, as well as the utilization of Inoculant A, B, or D. The inoculant comprised many strains of Lactobacillus plantarum and Enterococcus faecium. The level of direct messages (DMs) has experienced an increase. The pH of wilted silages was found to be higher than that of unwilted silages. Additionally, the application of Inoculants B on unwilted crops and Inoculants A, B, and D on wilted crops resulted in a decrease in silage pH as compared to the control group.

Higginbotham et al. (1998) identified five microbial inoculants, including *Lactobacillus plantarum* and *Pediococcus cerevisiae*. On corn silage *Propionibacterium acidipropionici*, *Pd. Cerevisiae*, in addition to prop. *Acidipropionici*, and *Prop*. With or without *Pd*. cerevisiae, adding *propionibacteria* had not much effect on pH.

Bolsen et al. (1996) determined the effects of a strain of *Propionibacterium shermanii*, applied with and without lactic acid bacteria (LAB), and discovered that the pH and acetic acid of all treatments are decreased, while lactic acid is increased. However, propionic acid was not detected in the control or LAB samples, while it was discovered in all other parameters.

Santos et al. (2009) investigated the chemical composition and nutritional content of sugar cane treated with *L. buchneri* at the time of ensiling. The dry matter percentage, crude protein, crude ash, ADF, and NDF all decreased. While only crude ash increased after 90 days of ensiling with *L.buchneri*, but crude protein, ADF, and NDF decreased.

Xu et al. (2019) looked at the microbial population and metabolome of corn silage treated with *homofermentative Lactobacillus plantarum* and *heterofermentative Lactobacillus buchneri*. To treat chopped whole-crop maize, deionized water, Lactobacillus buchneri, or Lactobacillus plantarum were utilized. Three replicates of each treatment were utilized to store the treated maize in vacuum-sealed polyethylene bags for 90 days. Acetic acid increased in *Lentilactobacillus buchneri* but decreased in Lactobacillus plantarum, although pH increased in both groups. Aerobic stability, propionic acid, yeast, and mold levels have all dropped.

Dreihuis et al. (1999) assessed maize silage inoculated with varying concentrations of *Lactobacillus buchneri* (1×10^{3}, 1×10^{4}, 1×10^{5} and 1×10^{6}). After inoculation, silage

showed an increase in acetic acid with inoculum levels above 1×10^4 and in DM and pH with inoculum levels above 1×10^5. Inoculation decreased yeast survival during the anaerobic storage phase and inhibited yeast growth when silage was subjected to O2, resulting in a significant improvement in the silage's aerobic stability.

Saarisalo et al. (2007) tested nine Lactic Acid Bacteria (*LAB*) strains for use as grass silage inoculants. Four promising strains were selected based on their growth rate, pH lowering ability, and ammonia-N production. The efficiency of the selected strains was evaluated using an ensiling experiment performed in the lab. All tested inoculants improved silage quality, whereas Timothy-meadow fescue silage caused pure lactic acid fermentation. Because the most promising strains can only be examined in long and onerous ensiling methods, rapid LAB strain analysis is useful for more systematic product development of commercial inoculant preparations.

Agarussi et al. (2019) investigated the fermentative profiles and microbial populations of alfalfa silages, both wilted and non-wilted, with and without the addition of an inoculant. The study employed a factorial design with three factors, each having two levels, resulting in a total of six fermentation phases. The alfalfa had a period of mild wilting lasting six hours, resulting in an elevation of the dry matter content. Lactic acid bacteria (LAB) were extracted from alfalfa fodder and silage following different fermentation durations. After the extraction of DNA, the amplification of 16S rRNA gene sequences was performed using the polymerase chain reaction (PCR) technique. The phenomenon of wilting has been observed to result in decreased levels of pH, NH4+, and acetic acid in silages. The process of wilting resulted in an increase in the type of lactic acid bacteria (LAB) present, but did not lead to a significant change in their quantity. The species Lactobacillus plantarum had the highest prevalence in both non-wilted and wilted silages.

Zielińska et al. (2015) investigated the effectiveness of three *lactobacilli* strains in the ensiling of alfalfa (*Medicago sativa L.*) for feed. The presence of molds, *Clostridium perfringens*, and *Listeria sp*. in alfalfa ensiling was reduced due to the use of bacterial inoculants. All the inoculants used for the treatment of silages, except for LPC, effectively inhibited the development of *Salmonella sp*. and *Escherichia coli*. The control silage exhibited the lowest level of aerobic stability. However, the application of bacterial inoculants resulted in a twofold increase in aerobic stability. The silage exhibiting the

highest levels of acetic acid, propionic acid, and 1, 2-propanediol was treated with LB inoculant in order to enhance its stability. The presence of antibiotic-resistance genes in lactic acid bacteria highlights the need of investigating the impact of bacterial inoculants on physicochemical and microbiological characteristics.

As seen in the above studies, both urea addition and inoculant addition caused significant changes on silage quality and resulted in an increase in quality characteristics. For this reason, in this thesis study, the effects of encapsulated urea and inoculants and their combinations in addition to corn silage were investigated.

CHAPTER 2

MATERIALS AND METHODS

In this thesis two experiment were carried out. The first study was carried out to investigate the effects of urease inhibitors urea and normal urea addition on chemical composition, silage quality properties, aerobic stability and microbiological properties of maize silages. The second study was designed to investigate effects of bacterial inoculant and urea addition to maize silage on chemical composition, silage quality properties, aerobic stability and microbiological properties of silages.

2.1. Material of silages

Corn plants were obtained Erciyes University Agricultural Research and Application Center (ERUTAM) located in Kayseri Turkiye at the coordinates of 06 57046" S and 35◦ 41031" W, 623 m altitude. Corn plants were harvested after 90 days after sowing, when kernels reached the dough stage about 290-310 g/kg of dry matter. Maize plants were harvested and chopped a silage maker (Fimaks Co, Bigdrum 2200, Bursa, Turkiye).

Granule urea and urease inhibitors urea (N-(n-tertbutyl) thiophosphoric triamide (NBPT) called "superinci" produced by Gubretaş AŞ. İstanbul, Turkiye and contained 46 % N and polymer coated slow release urea for ruminants (PCRU), distributed by Royal A.Ş., Kayseri.

Inoculant mixture Silamix (Royal A.Ş. Kayseri, Turkiye) and contained 3 bacteria and two enzymes were: L*actobacillus buchneri1x10^{14}Cfu/kg, Lactobacillus plantarum 8x10^{12}Cfu/kg*, and E*nterococcus faecium 2x10^{12}Cfu/kg* and enzymes; xylanase (endo-1.3-beta-xylanase) 2500000 FXU-W/kg and alpha amylase (1,4-alpha-amylase) 2000000 FAU-F/kg.

Corn plant was harvested at the time of pulping and urea (U), urease inhibitors urea and bacterial inoculant added groups were prepared. For this purpose, two experiments were planned. In the first study, the groups were formed as follows: 1: Control (silage without urea and inoculant addition), 2: Addition of 1% granule urea to corn silage, 3: Addition of 1% NBPT urea (PlU, (%46 N contained, Gubretaş AŞ. İstanbul, Turkiye) for plant fertilization, 3: 1% polymer coated urea for ruminants (PCRU, Royal A.Ş., Kayseri) were added. In the second study, 4: 0.05% inoculant (IN, Silamix, Royal A.Ş. Kayseri, Turkey) was added to the silage, 5: 0.1% IN was added to the silage, and 6: 0.2% IN was added to the silage. In addition, urea and inoculant combinations were created for silage.

2.2 Methods

2.2.1. Experimental design and treatments

The experimental design was completely randomized design with 7 treatments, and 5 replications. The experimental groups as follows:

Experiment 1:

1: Corn silage (control, C, no additive),

2: Urea (46% N) 1% addition

3: NBPT urea for plant fertilization, 1% addition (NBPTU)

4: Polymer slow release urea for ruminants nutrition, 1% addition (PSRU)

Experiment 2:

This experiment was designer 2x2 factorial arrangement, two types urea and inoculant addition.

1: Control (C, no inoculant addition)

2:Addition of Inocunalt 0.05% (L*actobacillus buchneri1x10^{14}Cfu/kg, Lactobacillus plantarum 8x10^{12}Cfu/kg,* and E*nterococcus faecium 2x10^{12}Cfu/kg* and enzymes; xylanase (endo-1.3-beta-xylanase) 2500000 FXU-W/kg and alpha amylase (1,4-alpha-amylase) 2000000 FAU-F/kg).

3: Addition of Inocunalt 0.1%

4: Addition of Inocunalt 0.2%

and combinations:

1: Normal urea addition %1 –no inoculant

2: Normal urea addition +inoculant

3: NBPT urea – no inoculant

4: NBPT urea + inoculant

The inoculant mixture was diluted in 100 mL distilled water and applied uniformly (sprayed 0.5 1, and 2 mL/kg chopped corn) according to the manufacturer's recommendations. The corn silage material were filled and pressed strictly in 2 liters glass jars.

2.3. Chemical analysis

2.3.1. Determination of Weende analyses, NDF and ADF composition

The silage samples' Weende analyses were done according to AOAC (2012). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyzes were done according to methods by using Ankom fiber analyses equipment. All samples were ground with a 0.5-1 mm sieve (Foss, The KN 295 Knifetec, Gerber instruments Co, Swiss) before chemical analyses.

2.3.2. Determination of PH

To determine pH values, 100 ml of distilled water to 25 g of silage sample used in a glass container then mixed with blender for 5 minutes. Silage pH were determined by using pH meter (Mettler Toledo, SevenCopact) via insert to mixture.

2.3.3. Determination of dry matter

The dry matter ratio was calculated by differencing the percentage change in the starting weight by the wet weight of the silage samples. They were reweighed after being dried at 70°C for 72 hours in oven (Nüve, KD 400, Ankara, Turkiye) to stabilize their weight.

2.3.4. Determination of crude protein

The amount of nitrogen (N) was determined by the Velp protein analyzer (Nitrogen analyzer NDA 702, Italy). This amount multiplied by the coefficient of 6.25 and crude protein (HP) ratio were calculated. Where samples were produced with an average weight of 0.400 mg and placed in a specific aluminum sheet before being placed in the device assigned location.

2.3.5. Determination of crude ash

Dried and ground 1 g sample of silage was weighted and burned for 4 hours at 550 °C. After cooling, the samples were reweight again, and the percentage of weight difference between the two weights was calculated to measure the amount of raw ash present in ash furnace (Protherm, RLF, 120/6, Turkiye).

2.3.6. Determination of crude fat

To determine crude oil of samples, 3.5-4 g samples of the feeds were obtained, mashed up, and then put through a 1mm filter in order to extract the oil using petroleum ether. The amount of crude oil in the feed were calculated by calculating the percentage of the difference after the feeds were dried at 105 °C and weighed again with the help of Velp fat extractor (SER-148, Italy).



Figure 2.1. Crude oil measurement and detector

2.3.7. Determination of neutral detergent fiber (NDF)

The dried feed sample through 0.5g 1 mm sieve were treated with Ankom NDF solution. The thoroughly washed samples were dried at 105 °C for 2 hours. After the analysis, the NDF ratio were determined by taking the difference between the dried and weighed feeds.

2.3.8. Determination of acid detergent fiber (ADF)

A sample of ground feed, 0.5 g in size to pass through a 1 mm sieve, were treated with ADF solution. The thoroughly washed samples were dried at 105 °C for 2 hours. ADF ratio were determined by taking the difference between the dried and weighed feeds after the analysis.

2.3.9. Determination of water soluble carbohydrate (WSC)

The silages WCS contents were determined according to Dubois et al. (1956). In this method, the liquid extractions were prepared with 40 g silage to be put into a beaker and added 360 ml distilled water, than mixed with a blander, then the liquid were filtered through Whatman 54 filter paper and then centrifuged. WSC concentrations were determined by spectrophotometer.

2.3.10. Determination of aerobic stability

Aerobic stability of silages were determined according to Ashbell et al. (1991). For this purpose, temperature changes were measured by a temperature probes (TMC6-HD)

placed in the silage material. Lactic acid (LA) ratio were determined by Lepper's methods (Akyildiz, 1986).

2.3.11. Determination of organic acids

Acetic, propionic and butyric acids were analyzed in a gas chromatograph (GC) with an capillary column (30 m × 0.25 mm × 0.25 μm, Restek), in a Shimadzu GC-2010+ (Kyoto, Japan). The GC was equipped with FID, over a temperature range of 45–230°C.

2.4. Determination of *in vitro* gas production

To determine of *in vitro* gas production, Hohenheim University in Germany and named Hohenheimer Futterwert Test (HFT) or Hohenheim feed test was used (DLG., 1981). According to method production of CO_2 and CH_4 that occurs as a result of 24-hour incubation at 39°C of feeds with rumen fluid in the incubator. Total gas production in syringe were determined and after 24 h produced gas injected to methane detector and methane gas determined as percentage.

The rumen fluids obtained from a commercial slaughterhouse operating in the Kayseri, whose feeding and disease history are known. About 10 kg rumen content mixture (fluid and sold part) were moved to the laboratory quickly with a nylon thermos container, and filtered to use. An artificial saliva in a 2: 1 ratio and fermentation liquid were prepared. The mixture obtained was taken into 30 ml syringes preheated at 39°C and kept in a 39°C water bath during the incubation period. The liquids prepared for all treatments were subjected to 24-hour incubation and the gas production amounts (recorded at 6, 12 and 24 h) were determined.

From these data (24 h total gas production), metabolisable energy (ME), net energy lactation (NEL) values and dry matter and organic matter digestibility of silages were determined.

Macro mineral solution: 5.7 g Na_2HPO_4 + 6.2 g KH_2PO_4 + 0.6 g $MgSO_4$ $7H_2O$ + It is dissolved in pure water and complete to 1000 ml.

Micro mineral solution: 13.2 g $CaCl_2$ $2H_2O$ + 10 g $MnCl_2$ $4H_2O$ + 1.0 g $CoCl_2$ $6H_2O$ + 8.0 g $FeCl_3$ 6 H_2O + It is dissolved in pure water and complete to 1000 ml.

Buffer solution: 39 g Na HCO_3 + 4 g (NH_4) HCO_3 + It is dissolved in pure water and completed to 1000 ml.

Resazurin solution: 100 mg dissolve resazurin in pure water and makeup to 100 ml.

Reduction solution: It is prepared fresh in every work. First 1.99 ml of 1 N NaOH solution is put into 47.45 ml of distilled water, 285 mg of Na2S 7HO is added.

The solutions prepared as described above were put into the woulf bottle in the amount and order given below.

474.50 ml distilled water + 0.12 ml micro mineral solution + 237.23 ml buffer solution + 237.23 ml macro mineral solution + 1.22 ml resazurin solution + 49.44 ml reduction solution.

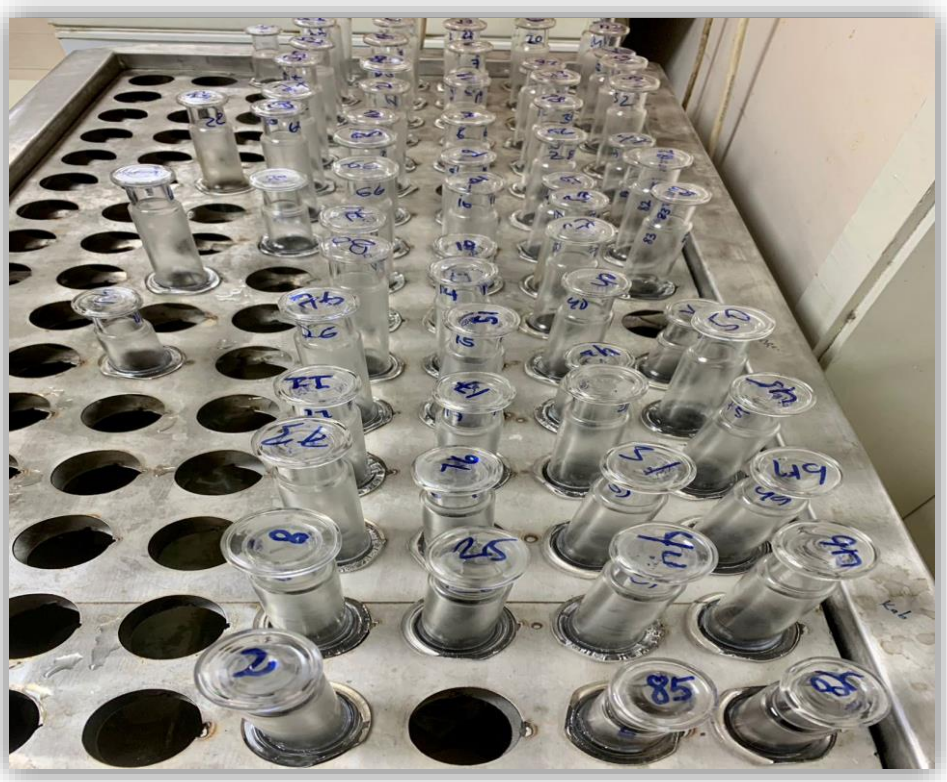


Figure 2.2. Incubation of prepared rumen fluid

2.4.1. Determination of methane gas percentage of corn silage

The percentage amount of methane (CH_4) gas formed as a result of fermentation in vitro in the total gas produced was determined using the methane detector (Picture 3.4).



Figure 2.3. Methane gas detect

2.5. Calculation of OMD and ME content corn silage

After the gas production amounts of plant by-products were determined, OMD (%), ME (MJ / kg), and NEL (MJ / kg) values were calculated according to Menke (1979) and shown below using the equations below.

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP
OMD (% DM) = 14.88 + 0.889 GP + 0.45 CP + 0.0651
NEL (MJ/kgDM) = 0.101 GP + 0.051 CP + 0.112 EE

2.6. Evaluation of data

The effects of different urea types and inoculant concentrations on the investigated parameters were determined by analysis of variance in SPSS.22 program One Way Anaova and General Linear Model for multivariation test. To determine effects combinations of different urea types and inoculants a factorial arrangenment (2x2) analyses were done with usingh General Linear Model in SPSS program. Duncan's multiple comparison tests were used to separate the source of the difference between the means. Significance level was accepted $P<0.05$.

CHAPTER 3

RESULTS

Corn silage used in the study were analyzed for pH, dry matter, crude protein, crude oil, and crude ash using the We require analysis technique at the Feeds and Animal Nutrition laboratories in the Department of Animal Science in the Faculty of Agriculture at Erciyes University. After that, gas production kinetics were determined.

In this study, two experiment were carried out as effects of different type urea and inoculant addition. Their results are presented separately below.

3.1. Effects of encapsulated urea addition to corn silage

In the experiment, three types urea were used.

Effects of addition of normal urea and urease inhibitors urea (NPBTU) and polymer coated urea (PCRU) on pH, dry matter, crude protein, and fat ADF and NDF values of corn silage showed in table 3.1. There were no differences among the groups in terms of pH, dry matter, crude fat, ash, and NDF content. However, protein concentration of all urea groups were higher than control group ($P<0.01$) and NPBTU group's CP ratio was higher than urea and PCRU groups. The ADF concentration in C group was higher than urea added groups ($P<0.05$). Water soluble carbohydrate (WSC) content of C and NPBTU groups were significantly higher than urea and PCRU groups ($P<0.01$).

Table 3.1. Effects of urea type on pH, dry matter, ADF, NDF, protein, and fat values of corn silage quality

Groups	Control	Urea	NPBTU	PCRU	P
pH	4.14±0.07	4.56±0.24	4.51±0.12	4.47±0.16	0.225
DM, %	31.82±0.36^{b}	33.32±0.47^{a}	32.58±0.46^{ab}	33.05±0.17^{a}	0.055
CP, %	7.05±0.19^{c}	15.63±0.18^{b}	16.68±0.18^{a}	15.88±0.28^{b}	0.001**
CF, %	1.44±0.04	1.47±0.04	1.35±0.08	1.46±0.05	0.493
CA, %	9.44±0.32	8.90±0.08	9.18±0.08	9.20±0.26	0.442
ADF, %	29.65±0.31^{a}	27.71±0.71^{b}	26.74±0.71^{b}	27.66±0.58^{b}	0.013*
NDF, %	40.72±1.53	44.01±1.09	42.94±1.31	44.33±1.36	0.240
WSC, %	23.44±0.53^{a}	21.07±0.40^{b}	24.33±0.21^{a}	20.17±0.59^{b}	0.001**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, DM: dry matter, CA: crude ash, CP: crude protein, CF: crude fat, ADF: acid detergent fiber, NDF: neutral detergent fiber, WSC: water soluble carbohydrate, NPBTU: urease inhibitor urea, PCRU: polymer coated urea for ruminants, P: probability, *:P<0.05, **P<0.01.

Effect of addition of urea types (NPBTU: urease inhibitor urea, PCRU: polymer coated urea for ruminants) to the corn silage quality on aerobic stability and microbiology shown in table 3.2. According to the table there were strongly positive statistically significant differences between the control group and urea types (P<0.01). In term of aerobic stability DM the highest value obtained from the plan encapsulated urea, while the lowest obtained from control group. The difference between the groups in the term of aerobic stability pH, carbondioxyde, aerobic stability yeast the highest value obtained from encapsulated rumen. In terms of aerobic stability mold and mold, the highest value obtained from control. In terms of yeast and lactobacillus, the highest value obtained from urease inhibitor urea.

Table 3.2. Effects of urea type on aerobic stability and microbiology of corn silage quality

	Control	Urea	NPBTU	PCRU	P
AS, DM (%)	31.71±0.16^{b}	33.07±0.13^{a}	33.23±0.37^{a}	32.63±0.30^{a}	0.002**
AS pH	4.80±0.01 ^{c}	4.87±0.01 ^{b}	4.83±0.01 ^{c}	5.05±0.01 ^{a}	0.001**
AS yeast (log10 kob/g DM)	5.61±0.02^{c}	5.85±0.00^{b}	4.45±0.01^{d}	5.97±0.00^{a}	0.001**
AS Mold (log10 kob/g DM)	3.53±0.01^{a}	2.38±0.97^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.001**
Yeast,(log_{10} kob/g DM)	4.05±0.00^{d}	4.89±0.02^{a}	4.15±0.01^{b}	4.09±0.01^{c}	0.001**
Lactobacilli, (log_{10} kob/g DM)	3.91±0.02^{c}	4.83±0.01^{a}	4.62±0.02^{b}	4.66±0.01^{b}	0.001**
Mold, (log_{10} kob/g DM)	2.50±0.00^{a}	1.64±0.68^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.001**
Carbondioxyde (g/kg KM)	6.15±0.17^{b}	4.40±0.07^{d}	5.32±0.06^{c}	8.22±0.23^{a}	0.001**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, DM: dry matter, AS: Aerobic stability, P: probability, NPBTU: urease inhibitor urea, PCRU: Encapsulated Rumen, P: probability, **P<0.01

Effects of different urea type addition to corn silage on acetic, butyric and propionic acid concentrations given in table 3.3. Addition of urea to corn silage did not effect on acetic, butyric and propionic acid concentration (P>0.05).

Table 3.3. Effects of urea type on organic acids of corn silage

Groups	Acetic acid, g/kg DM	Propionic acid, g/kg DM	Butyric acid, g/kg DM
Control	17.16±2.84	3.81±1.87	8.67±3.83
Urea	18.32±4.11	8.77±4.04	7.04±3.29
NPBTU	24.81±3.87	5.39±0.79	10.94±4.75
PCRU	20.21±5.34	6.04±0.91	6.22±2.34
P	0,615	0.544	0.797

P: probability.

Effect of addition of corn silage with urea on gas and methane production parameters shown in Table 3.4. According to table, there were significant differences between treatment (P<0.05). In term of gas production 6, 12, and 24 hour, the highest value obtained from control group, while the lowest value obtained from encapsulated rumen.

However, in term of CH4 the highest value obtained from encapsulated rumen, while lowest value control group.

Table 3.4. Effects of corn silage added with urea type on total gas (ml) and methane production (%) parameters

	Measurement hours			
Groups	6	12	24	CH_4
Control	39.25±1.38[a]	51.08±1.54[a]	66.58±1.51 [a]	8.85±0.37 [b]
Urea	36.70±0.83[ab]	45.20±1.31[b]	60.00±3.17 [b]	9.86±0.55 [ab]
NPBTU	34.60±1.29[b]	44.10±1.57[b]	58.60±2.31 [b]	9.86±0.48 [ab]
PCRU	34.50±0.42[b]	43.60±1.04[b]	57.30±1.01 [b]	10.72±0.15 [a]
P	0.018*	0.004**	0.023*	0.034*

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, AS: Aerobic stability, P: probability, NPBTU: urease inhibitor urea, PCRU: Encapsulated Rumen, P: probability, *:P<0.05, **P<0.01, CH_4: methane.

Effect of addition of corn silage with urea on organic digestibility show in Table 3.5. According to the table there were significant between GP, ml/24saat the highest value obtained control group, while the lowest obtained from encapsulated rumen (P<0.05). However, there were no significant difference between other groups (P>0.05).

Table 3.5. Effects of corn silage added with urea type on GP, ml/24saat, OMD%, ME, Mj/kg DM, NEL, ME, kcal/kg).

Item	Control	Urea	NPBTU	PCRU	P
GP, ml/ 24saat	36.58±1.51**a**	30.00±3.17**b**	28.60±2.31**b**	27.30±1.01**b**	0.023*
OMD, %	51.19±1.39	49.16±2.77	48.41±1.99	46.89±0.78	0.423
ME, MJ/kg DM	7.58±0.21	7.17±0.43	7.04±0.31	6.82±0.12	0.289
NEL, MJ/kg DM	4.22±0.16	3.99±0.32	3.89±0.23	3.73±0.09	0.432
ME (kcal/kg)	1803.17 ±50.28	1706.80±101.22	1675.60±73.12	1622.80±29.36	0.294

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: Encapsulated rumen type urea, GP: gas production, OMD: organic matter digestibility, ME" Metabolisable Energy, NEL: Net energy lactation, P: probability, P: probability, *:P<0.05.

3.2. Effects of inoculant addition to corn silage

Effects of inoculants addition to corn silage on pH, dry matter, crude ash, protein, fat, and ADF and NDF values were given in Table 3.6. Crude protein concentration of inoculant supplemented group 0.5% and 1% was higher than control group. But there is no difference between inoculant 0.5%, 1% groups, and inoculant 2%, crude fat ratio, inoculant 0.5% was higher than those groups control, urea1% and 2%. However there is no difference in a fat concentration of those groups control, urea1% and 2%. ADF concentration control group was higher than different percentage of inoculant addition inoculant 0.5%, 1% and 2%. Although there is no difference in inoculant supplemented groups 0.5%, 1% and 2% (P>0.05) .NDF ratio inoculant 0.5% and 2% was higher than the control and inoculant 1%, and inoculant 1% was higher than control groups. But there is no significant difference in inoculant 0.5% and 2 % (P<0.05).

Table 3.6. Effects of inoculants addition to corn silage on pH, dry matter, crude ash, protein, fat, ADF and NDF values

Groups	Control	IN 0.5%	IN 1%	IN 2%	P
pH	4.14±0.07	4.38±0.11	4.38±0.07	4.14±0.03	0.061
Dry Matter	31.82±0.36	32.68±0.50	32.29±0.40	32.61±0.78	0.584
Crude ash	9.44±0.32	9.39±0.14	9.29±0.08	9.27±0.13	0.937
Crude protein	7.05±0.19^{b}	7.84±0.12^{a}	7.82±0.12^{a}	7.47±0.07^{ab}	0.003**
Crude fat	1.44±0.04^{b}	1.76±0.06^{a}	1.49±0.05^{b}	1.57±0.04^{b}	0.001**
ADF	29.65±0.31^{a}	27.77±0.31^{b}	27.73±1.27^{b}	27.35±0.73^{b}	0.134
NDF	40.72±1.53^{c}	43.85±0.44^{a}	42.57±0.64^{b}	43.12±1.29^{a}	0.243
WSC	23.44±0.53	22.51±0.50	18.53±0.52	19.70±0.47	0.001**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: Encapsulated rumen type urea, P: probability, **P<0.01.

Effects of inoculants addition on aerobic stability and microbiology of corn silage quality was given in Table 3.7. Aerobic stability pH of inoculant 0.5% and 1% group was higher than the control and inoculant2%. There is no different group inoculant addition 0.5% and 1%. Also there is no difference between control group and inoculant 2% group. (P<0.05). Aerobic stability yeast, control group was higher than addition of inoculant 2% whıle addition of inoculant 2% are higher than those supplemented group of 0.5% and 1%. There is no significance difference between addition of inoculant 0.5% and 1%.

Aerobic stability Mold, control group was higher than inoculant 2% group, and inoculant 2% group was higher than inoculant 1% group, also inoculant 1% group was higher than 0.5% group (P<0.05).Yeast, control group was higher than inoculant 0.5% group, Although inoculant 0.5% group was higher than those inoculant addition groups inoculant1% and 2%, but there is no significance difference between inoculant1% and 2% groups. *Lactobacilli,* addition of inoculant 1% group is higher than control, and control groups is higher than inoculant 2% group, also inoculant 2% is higher than inoculant 0.5% group. (P<0.05). Mold, inoculant 0.5% group was higher than inoculant 2% group, and inoculant 2% group, was higher than inoculant 1% also inoculant 1 % group was higher than control group. Co2. Those groups control and inoculant 0.5% Was higher than both inoculant 1 and 2 % group, although control and inoculant 0.5% groups there is no significance between them as same as the inoculant 1 and 2 % group there is no significance between these groups.

Table 3.7. Effects of different inoculants on aerobic stability and microbiology of corn silage quality.

Item	Control	IN-0.5%	IN-1%	IN-2%	P
AS DM,%	31.71±0.16^{b}	33.00±0.53^{a}	33.35±0.29^{a}	32.64±0.36^{b}	0.015*
AS pH	4.80±0.01^{ab}	4.80±0.00^{b}	4.83±0.01^{a}	4.81±0.00^{ab}	0.071
CO_2, g/kg DM	6.15±0.17^{a}	4.35±0.24^{c}	4.38±0.12^{c}	5.29±0.08^{b}	0,001**
AS yeast, log_{10} kob/g DM	5.61±0.02^{a}	4.32±0.01^{d}	4.55±0.01^{c}	4.86±0.02^{b}	0,001**
AS mold, log10 kob/g DM	3.53±0.01^{a}	1.87±0.76^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0,001**
Yeast,log_{10} kob/g DM	4.05±0.00^{b}	3.75±0.03^{d}	4.33±0.01^{a}	3.94±0.03^{c}	0,001**
Lactobacilli, log_{10} kob/g DM	3.91±0.02^{d}	4.44±0.01^{a}	4.29±0.01^{c}	4.35±0.01^{b}	0,001**
Mold, log_{10} kob/g DM	2.50±0.00^{a}	1.55±0.64^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0,001**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, AS: Aerobic stability, NPBTU: urease inhibitor urea, PCRU: Encapsulated rumen type urea, P: probability, *:P<0.05, **P<0.01.

Effects of inoculants addition on acetic, butyric and propionic acid concentrations are given in table 3.8. Addition of different percentage of inoculants, (0.05%, 0.1% and 0.02%) to corn silage did not effect on acetic, butyric and propionic acid concentration (P>0.05).

Table 3.8. Effects of different Inoculants on Organic acids of corn silage quality.

Groups	Acetic acid ml/kg	Propionic acid	Butyric acid
Control	17.16±2.84^{ab}	3.81±1.87	8.67±3.83
IN -0.5%	14.24±2.09^{ab}	7.27±1.35	4.67±1.46
IN-1%	21.69±3.19^{a}	7.56±1.64	5.64±2.85
IN-2%	10.86±1.44^{b}	3.43±0.72	0.96±0.23
P	0.056	0.142	0.275

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: Encapsulated rumen type urea, P: probability.

Effects of different percentage of inoculants (inoculant 0.5%, inoculant 1% and inoculant 2%) addition to corn silage on gas production at 6, 12, and 24 hours and methane gas ratio are given in table 3.9. Gas production at 6h in inoculant 2% was higher than those groups control group inoculant 0.5%, inoculant 1% groups. But there is no difference between control group inoculant 0.5%, inoculant 1% groups ($P<0.05$). In the 12 hours inoculant 2% was higher than control group inoculant 0.5%, inoculant 1% groups ($P<0.05$). In 24 hours did not effect on gas productıon ratio ($P>0.05$). Methane in inoculant 2% was higher than control group while the control group was higher than inoculant 0.5%, inoculant 1% groups but inoculant 0.5%, inoculant 1% there is no significance between them ($P<0.05$).

Table 3.9. Effects of corn silage added with different inoculants on gas production parameters

Groups	6 hours	12 hours	24 hours	CH4
Control	39.25±1.38^{b}	51.08±1.54 ^{b}	66.58±1.51	8.85±0.37 ^{b}
IN -0.5%	43.20±0.44^{b}	55.10±0.33^{ab}	71.40±0.58	7.54±0.30 ^{c}
IN-1%	41.40±0.80^{b}	54.30±0.97 ^{b}	70.40±0.95	7.98±0.15^{bc}
IN-2%	53.50±3.14^{a}	60.88±4.20 ^{a}	71.88±4.85	10.73±0.51 ^{a}
P	0.000**	0.025*	0.299	0.000**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: Encapsulated rumen type urea, P: probability, *:$P<0.05$, **$P<0.01$.

Effects of different percentage of inoculants (inoculant 0.5%, inoculant 1% and inoculant 2%) addition to corn silage on organic digestibility show in Table 3.10. According to the table there were no significance gas production, organic matter digestibility,

Metabolisable Energy (ME), and net energy lactation (NEL) values of inoculant additive groups (P>0.05)

Table 3.10. Effects of addition of inoculant on GP, ml/24saat, OMD, ME and NEL values of corn silages

Groups			NEL, MJ/kg	
	GP, ml/24saat	OMD, %	DM	ME(Kcal/Kg)
Control	36.58±1.51	51.19±1.39	4.22±0.16	1803.17±50.28
IN -0.5%	41.40±0.58	55.82±0.56	4.78±0.06	1970.20±20.07
IN-1%	40.40±0.95	54.92±0.84	4.64±0.09	1937.40±30.92
IN-2%	41.88±4.85	56.07±4.31	4.79±0.49	1980.25±156.76
P	0.299	0.255	0.221	0.260

IN: inoculant, GP: gas production, OMD: organic matter digestibility, ME: Metabolisable Energy, NEL: Net energy lactation, P: probability.

3.3. Effects of urea type and inoculant combinations on silage quality characteristics

The effect of urea, encapsulated urea, and inoculant on pH, dry matter, ash, ADF, and NDF are shown in Table 3.11. According to this table, there is no significant difference in pH, dry matter, crude ash, ADF, and NDF between urea and encapsulated urea (p>0.05). However, there a significance different in fat and protein (P<0.05) in urea1%+IN1% and PCRU. The highest fat obtained the inoculant rumen while the lowest obtained the urea1%+IN1%. The interaction between the treatments was significantly affect the crude protein (P<0.05).

Table 3.11. Effect of urea, encapsulated urea and inoculant rumen addition on pH, DM, ash, ADF, NDF, and fat of corn silage

Groups	pH	DM, %	CA, %	CP, %	CF, %	ADF, %	NDF, %
Urea	4.53±0.12	33.48±0.24	8.86±0.10	15.98±0.13	1.51±0.04	27.18±0.37	43.20±0.67
PCRU	4.42±0.12	33.39±0.24	9.01±0.10	15.63±0.13	1.54±0.04	27.42±0.37	43.51±0.67
P	0.538	0.799	0.302	0.066	0.583	0.646	0.754
Inoculant addition							
No –IN	4.52±0.12	33.18±0.24	9.0±0.10	15.76±0.13	1.46±0.04^{b}	27.69±0.37	44.17±0.67
+IN	4.44±0.12	33.68±0.24	8.8±0.10	15.86±0.13	1.59±0.04^{a}	26.92±0.37	42.54±0.67
P	0.661	0.160	0.106	0.558	0.042*	0.163	0.106
Interaction							
Urea no-IN	4.56±0.18	33.32±0.34	8.90±0.14	15.63±0.18^{b}	1.47±0.06	27.71±0.53	44.01±0.95
Urea+IN	4.47±0.18	33.05±0.34	8.81±0.14	15.88±0.18^{ab}	1.46±0.06	27.66±0.53	44.33±0.95
PCRU- no IN	4.51±0.18	33.64±0.34	9.20±0.14	16.34±0.18^{a}	1.55±0.06	26.65±0.53	42.39±0.95
PCRU +IN	4.37±0.18	33.73±0.34	8.81±0.14	15.39±0.18^{b}	1.62±0.06	27.18±0.53	42.68±0.95
P	0.880	0.607	0.314	0.004*	0.493	0.589	0.988

DM: dry matter, CA: crude ash, CP: crude protein, CF: crude fat, ADF: acid detergent fibre, NDF: neutral detergent fibre, WSC: water soluble carbohydrate, NPBTU: urease inhibitor urea, PCRU: polymer coated urea, P: probability, *: P<0.05.

Effects of supplementation of urea, PCRU urea, urea1%+IN1%, and inoculants rumen to the corn silage and their interaction on aerobic stability and microbiology shown in Table 3.12. According to the table the urea increased aerobic stability km, aerobic stability yeast, and yeast value, but PCRU urea decreased in the CO_2 and aerobic stability pH ($P<0.05$). Addition of urea1%+IN1% increased aerobic stability pH, carbondioxyde, aerobic stability yeast, yeast, and lactobacilli ($P<0.05$). However, there was a significant difference between the treatments interaction ($P<0.05$), except aerobic stability DM value ($P>0.05$).

Table 3.12. Effect of urea, PCRU urea and inoculant addition on aerobic stability, CO_2, lactobacilli, yeast and mold lactobacilli, yeast and mold

Groups	Aerobic stability DM (%)	Aerobic stability pH	Carbondioxyde (G/Kg DM)	Aerobic stability yeast Log10 Kob/G DM	Aerobic stability mold Log10 Kob/G DM	Yeast, Log10 Kob/G KM	Lactobacilli, Log10 kob/G DM	Mold, Log10 kob/G DM
Urea	33.45±0.21^{a}	4.87±0.00^{b}	4.42±0.10^{b}	5.72±0.00^{a}	1.19±0.46	4.73±0.01^{a}	4.70±0.01	0.82±0.32
PCRU	32.77±0.21^{b}	4.97±0.00^{a}	6.02±0.10^{a}	5.48±0.00^{b}	1.07±0.46	4.15±0.01^{b}	4.70±0.01	0.74±0.32
P	0.034*	0.000**	0.000**	0.000**	0.858	0.000**	0.657	0.861
Inoculant addition								
No-IN	32.85±0.21	4.96±0.00^{a}	6.31±0.10^{a}	5.91±0.00^{a}	1.19±0.46	4.49±0.01^{a}	4.75±0.01^{a}	0.82±0.32
+IN	33.37±0.21	4.88±0.00^{b}	4.13±0.10^{b}	5.30±0.00^{b}	1.07±0.46	4.39±0.01^{b}	4.65±0.01^{b}	0.74±0.32
P	0.091	0.000**	0.000**	0.000**	0.858	0.000**	0.000**	0.861
Interaction								
Urea no-IN	33.07±0.29	4.87±0.01^{b}	4.40±0.14^{b}	5.85±0.01^{ab}	2.38±0.65^{a}	4.89±0.02^{a}	4.83±0.01^{a}	1.64±0.45^{a}
Urea+IN	32.63±0.29	5.05±0.01^{a}	8.22±0.14^{a}	5.97±0.01^{a}	0.00±0.65^{b}	4.09±0.02^{b}	4.66±0.01^{b}	0.00±0.45^{b}
PCRU- no IN	33.82±0.29	4.87±0.01^{b}	4.44±0.14^{b}	5.59±0.01^{ab}	0.00±0.65^{b}	4.57±0.02^{b}	4.57±0.01^{b}	0.00±0.45^{b}
PCRU +IN	32.92±0.29	4.90±0.01^{b}	3.83±0.14^{b}	5.00±0.01^{b}	2.15±0.65^{a}	4.21±0.02^{b}	4.73±0.01^{ab}	1.48±0.45$_{a}$
P	0.441	0.000**	0.000**	0.000**	0.003*	0.000**	0.000**	0.003*

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: PCRU rumen type urea, P: probability, *: P<0.05, **P<0.01. DM: dry matter.

Effect of urea, PCRU urea, urea1%+IN1%, and inoculant rumen on organic acids of corn silage are shown in table 3.13. According to the table there is no significance difference between urea, PCRU urea, urea1%+IN1% and inoculant rumen and their interaction (P>0.05).

Table 3.13. Effect of urea, PCRU urea, urea1%+IN1%, and inoculant rumen on organic acids of corn silage

Groups	Acetic Acid	Propionic Acid	Butyric Acid
Urea	16.89±3.24	7.08±1.49	12.27±2.85
PCRU	18.99±3.24	4.82±1.49	7.96±2.85
P	0.653	0.302	0.301
Inoculant addition			
No-IN	19.27±3.24	7.40±1.49	6.63±2.85
+IN	16.62±3.24	4.50±1.49	13.60±2.85
P	0.571	0.188	0.103
Interaction			
Urea no-IN	18.32±4.58	8.77±2.11	7.04±4.03
Urea+IN	20.21±4.58	6.04±2.11	6.22±4.03
PCRU- no IN	15.47±4.58	5.38±2.11	17.50±4.03
PCRU +IN	17.76±4.58	3.61±2.11	9.70±4.03
P	0.966	0.825	0.399

[a,b,c,d]: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: PCRU rumen type urea, P: probability.

Effect of urea, PCRU urea and inoculant rumen addition and their interaction on rumen gas and methane production are given in Table 3.14. According to the table in the term of urea and PCRU urea, affected of rumen gas and methane production in the value of 12 hour (P<0.05). Addition of urea1% +IN1% and inoculant rumen on rumen gas and methane production were significant effect (P<0.05). Urea1%+IN1% had a significantly lower rumen gas and higher methane production. The interaction between urea and EN urea *inoculant rumen had a significant effect on the rumen gas and methane production (P<0.05).

Table 3.14. Effect of urea, PCRU urea and inoculant addition on rumen gas and methane production

Groups	6 hours	12 hours	24 hour	CH4
Urea	36.40±0.37	45.40±0.62^{b}	60.05±1.18^{b}	10.14±0.23^{a}
PCRU	37.40±0.37	47.55±0.62^{a}	62.85±1.18^{a}	9.48±0.23
P	0.073	0.026*	0.114	0.055
Inoculant addition				
No-IN	35.60±0.37^{a}	44.40±0.62^{b}	58.65±1.18^{b}	10.29±0.23
+IN	38.20±0.37^{b}	48.55±0.62^{a}	64.25±1.18^{a}	9.33±0.23
P	0.000**	0.000**	0.004*	0.008*
Interaction				
Urea no-IN	36.70±0.52^{b}	45.20±0.88^{b}	60.00±1.67^{b}	9.86±0.32^{ab}
Urea+IN	34.50±0.52^{b}	43.60±0.88^{b}	57.30±1.67^{b}	10.72±0.32^{a}
PCRU- no IN	36.10±0.52^{b}	45.60±0.88^{b}	60.10±1.67^{b}	10.42±0.32^{a}
PCRU+IN	40.30±0.52^{a}	51.50±0.88^{a}	68.40±1.67^{a}	8.24±0.32^{b}
P	0.000**	0.001**	0.005**	0.000**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: PCRU rumen type urea, P: probability, *: P<0.05, **P<0.01.

Effects of addition of urea and inoculant combinations on GP, ml/24saat, OMD, ME and NEL values of corn silages are presented in table 3.15.

Table 3.15. Effects of addition of urea and inoculant combinations on GP, ml/24saat, OMD, ME and NEL values of corn silages

Groups	OMD, %	ME, MJ/kg DM	NEL, MJ/kg DM	ME(Kcal/Kg)
Urea	49.36±1.03	7.20±.16	4.02±.12	1713.10±37.55
PCRU	51.71±1.03	7.56±.16	4.29±.12	1799.00±37.55
P	0.126	0.127	0.124	0.126
Inoculant addition				
No-IN	48.03±1.03	7.00±.16	3.86±.12	1664.80±37.55
+IN	53.04±1.03	7.76±.16	4.45±.12	1847.30±37.55
P	0.003**	0.003**	0.003**	0.003**
Interaction				
Urea no-IN	49.16±1.45	7.17±.22	3.99±.17	1706.80±53.11
Urea+IN	49.56±1.45	7.22±.22	4.05±.17	1719.40±53.11
PCRU- no IN	46.89±1.45	6.82±.22	3.73±.17	1622.80±53.11
PCRU+IN	56.52±1.45	8.30±.22	4.85±.17	1975.20±53.11
P	0.006**	0.006**	0.006**	0.006**

^{a,b,c,d}: Differences between the averages are significant in the same column with different letter, NPBTU: urease inhibitor urea, PCRU: PCRU rumen type urea, P: probability, *: P<0.05, **P<0.01.

CHAPTER 4

DISCUSSION

4.1. Effects of urea addition to silage chemical composition

In this study, normal granule urea and encapsulated urea were used to improve corn silage quality. Encapsulation of urea significantly affected the chemical composition of the silages and did not affect the pH, DM, CF, CA and NDF ratios compared to the control group. The protein content was found to be significantly higher than the control group, which is entirely related to the increased N content in the silage. However, the effects of urea forms on the protein ratio of silage were not found significant among themselves. In addition, a significant decrease in ADF ratio was observed in silages with urea compared to the control group, but the differences between urea forms were not significant.

Although good quality silage can be made without adding any additives, substances such as grain feeds (barley, wheat, corn, etc.) and molasses are added to corn and sunflower silages as energy sources in order to accelerate fermentation, reduce silo losses and enrich them with nutrients (Konca et al., 2018; Büyükkiliç Beyzi et al., 2016). In addition, urea addition has been made since ancient times in order to increase the protein content of silage (Denek, et al., 2004; Yunus et al., 2000; Gómez-Vázquez et al., 2011; Demirel et al., 2003). However, when urea, one of the silage additives, is added to the silage, providing a good distribution and loss from the environment due to the volatility of N may be in question. Thanks to the technological advances developed in recent years, there are important publications that the slow dissolution of urea in both plant nutrition and animal nutrition increases the usefulness (Highstreet et al., 2010; Ceconi et al., 2015; Granja-Salcedo et al., 2019; Lira-Casas et al., 2019).

In this current study the aerobic stability DM the highest value obtained from the urease inhibitor urea, while the lowest obtained from control group. The difference between the

groups in the term of aerobic stability pH, carbon dioxide, aerobic stability yeast the highest value obtained from encapsulated rumen. In terms of aerobic stability mold and mold, the highest value obtained from control. In yeast and lactobacillus, the highest value obtained from urease inhibitor urea. Addition of urea to corn silage did not effect on acetic, butyric and propionic acid concentration. Gas production 6, 12, and 24 hour, the highest value obtained from control group, while the lowest value obtained from encapsulated rumen. However, in term of CH4 the highest value obtained from encapsulated rumen, while lowest value control group. There were significant between GP, ml/24saat the highest value obtained control group, while the lowest obtained from encapsulated rumen. However, there were no significant difference between other groups.

Hou et al. (2022) who obtained that the urea supplementation with alfalfa silage, 0.05%urea had a higher ADF value than 1%urea, also higher CP value than control and 0.5%urea, 1% urea had a higher pH. In another study (Sariçiçek and Kiliç, 2009) found that the corn silage added 1%urea ensiling 60 days, dry matter and NE significant decreased, butyric, acetic and lactic acid showed non-significant. The first six hours of the gas production were decreased, but the next 12 and twenty-24 hours were non-significant. Also it is reported (Demirel et al., 2003) that the quality characteristics of corn silage added 0.5%urea, had a significant increased CP, pH, and dry matter and decreased in propionic acid, while did not significant in ADF, NDF, butyric, and acetic acid value.

Santos et al. (2021) noted that the effect of sorghum silage added with different percentage of urea, adding urea at 0.5 and 1% concentrations may improve aerobic stability in sorghum silage. However, the use of 2% urea increases silage fermentation.

4.2. Inoculant effects on silage quality

In this current study inoculant were used to improve corn silage quality. Inoculant significantly affected the chemical characteristics of the silage and did not affect DM, pH, crude ash, AFD, and NFD. The crude fat and crude protein was significantly increased in silage with inoculant compared to the control group. Also inoculant did not significant affect organic acids, aerobic stability pH, carbon dioxide KM, and gas production.

Jin et al. (2015) reported that ability of L. buchneri-based inoculants to increase the aerobic stability of barley silage by producing acetic acid and reducing water soluble carbohydrates. Filya et al. (2007) noted that difference microbial inoculant increased

alfalfa silage characteristics by decreasing pH and changing fermentation of lactic acid. However, the positive effect on silage fermentation, inoculation with lactic acid bacteria did not increase in vitro dry matter digestibility. Nair et al. (2020) reviewed that the aerobic stability of corn silage was increased after inoculation with a mixture of L. hilgardii and L. buchneri. Gollop et al. (2005) found that LAB silage inoculants produce antibacterial activity was able to prevent harmful microorganisms from growing in the silage or the rumen. Khorvash et al. (2006) reported that inoculant and absorbent added to corn silage was increased the nutritional value of silage. Sucu and Filya, (2006) who found that homofermentative LAB inoculants increased early lactic fermentation, reducing pH, lower protein, and fermentation losses. However, homofermentative LAB inoculants reduced the aerobic stability of wheat silages. (da Silva Ávila et al., 2010) noted that the application of bacterial inoculants increased the microbiological characteristics, while decreased ethanol production in sugar cane silages.

Acosta Aragón et al.(2012) found that the microbial inoculant on hetero- and homofermentative lactic bacteria had a strong significant effect on corn silage quality in the term of lowering the pH and changing fermentation to lactic acid, decreasing butyric acid, ethanol, and significantly reducing DM losses. In another study, Hristov and McAllister (2002) found that the inoculants did not improve the DM degradability of barley silage, lower terminal pH and higher lactic acid concentrations may improve aerobic stability. Similar results observed by Addah et al. (2011) in corn and barley silage and inoculation had no effect on loses of DM and improve silage fermentation. Aksu et al. (2004) found that the bacterial inoculation increased corn silage fermentation and digestibility of DM and NDF.

Inocunlat concentration and type added to silages can significantly affect the degree of fermentation of silages (Muck et al., 2018). Silajlara katılan LAB bakteri konsantrasyonu ve LAB çeşidi silajın fermantasyon derecesini önemli derecede etkileyebilmektedir. Also, preactivation of bacteria added to silages increases their effectiveness. In a study (Bağcık et al., 2022), the addition of Preactivated LAB to silage reduced silage pH, ammonia nitrogen, cell-wall components, yeast count, and carbon dioxide production, as well as increasing lactic acid and residual water-soluble, compared to LAB stored in freez dry. They reported that it was significantly effective on carbohydrate and aerobic stability.

4.3. Effects of urea type and inoculant combination on silage quality

When the effects of inoculant addition to normal urea and polymer coated urea added silages were examined, it was determined that when only urea was added, its effects on pH, DM, Ash, protein, fat, ADF, NDF were not significant, but the inoculant addition changed the crude fat ratio of the silage but did not affect other parameters. When urea and probiotic combinations were examined, it was determined that the addition of PCRU type urea increased the crude protein level compared to other groups except urae+In. However, the increase in protein level here is thought to be partly coincidental. This result needs to be repeated with other experiments to confirm it. It was expected that adding urea and probiotic together could create a synergy and slow release could occur with coated urea. If a synergy occurs, there may be significant improvements in aerobic stability, energy values and organic matter usability of silage. As a matter of fact, in this study, thanks to this association, improvements were made in aerobic stability and increase in energy values. However, there was a slight increase in gas production values with the addition of inoculant. Bağcık et al. (2022) reported that LB addition with urea improved in cell wall degradation, crude protein, water soluble carbohydrate, and lactic acid concentrations and aerobic stability of corn silages. The authors claimed that addition of preactivated LAB and urea combination improved fermentation traits, nutritional quality and aerobic stability and methane production ratio in silages.

CONCLUSION AND RECOMMENDATIONS

Corn is one of the most widely used forage crops because it is easy to grow and reach, contains enough easily soluble carbohydrates for fermentation, but they are lower protein content that's why farmers they add urea as a source of protein content because of true protein is very expensive price in a market, Also some of the farmers use commercial inoculants to improve silage quality like good fermentation characteristics, organic acids, and microbiological characters.

Corn silage is commonly used as animal feed. As these are obtained as a supplement to the main product, they can provide positive improvements in livestock farming in reducing feeding costs and meeting the protein and energy needs of animals, improving economic benefits.

Animals can be supplied with nutrients by the use of these feeds in animal feeding, but they have been successful in reducing methane gas production. However, this study is thought to be a reference for other researchers.

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CURRICULUM VITAE

Name: Maryama Khalif MOHAMUD

Date of birth: 07/12/1996

Nationality: Somalia

Education information:

Graduated from the Veterinary Science, Department of Animal Husbandry, Somali National University, Somalia (2020-2021).

Contact information:

Tel. +252618321501 / +905528987897

Email. maryamakhalif1501@gmail.com

Languages:

Arabic: Normal

English: Good

Somalia: Excellent/mother language

Turkish: Normal

Experience:

Good experience in computer software, Excel, SPSS.

Good experience in animal farming, feed production and nutrient analysis.

Worked Somali meat company (SOMMEAT).

Founder of Hamuuntire Livestock products.

Scientific publications:

1. Effect of inoculant concentration on corn silage quality and in-vitro gas production in beef cattle. VII. International Congress on Domestic Animal Breeding, Genetics and Husbandry - 2023 (ICABGEH-23) September 18 - 20, 2023 Krakow, Poland.

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