EFFECTS OF DIFFERENT LEVELS OF ACACIA *(Robinia pseudoacacia)* TREE LEAVES AND MOLASSES ON ALFALFA SILAGE QUALITY, *IN VITRO* RUMEN FERMENTATION, METHANE PRODUCTION AND NUTRIENT DIGESTIBILITY

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SAĞLIK BİLİMLERİ ENSTİTÜSÜ
Graduate School of Health Sciences

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ABSTRACT

Effects Of Different Levels Of Acacia (*Robinia pseudoacacia*) Tree Leaves And Molasses On Alfalfa Silage Quality, *In Vitro* Rumen Fermentation, Methane Production And Nutrient Digestibility

Aims: This study was conducted to evaluate the effects of acacia tree leaves and molasses addition on alfalfa silage (AAS) fermentation quality, *in vitro* ruminal fermentation, nutrient digestibility, methane mitigation and ammonia nitrogen (NH_3-N) excretion. The study also intends to find an alternative and sustainable feeding source in the ruminant production system.

Material and methods: The 3rd cut of fresh alfalfa was chopped and randomly divided into eight groups: without additive control (C), 5% molasses (M5), 3% acacia leaves (A3), 6% acacia leaves (A6), 12% acacia leaves (A12), and combination of acacia leaves and molasses M5+A3, M5+A6, and M5+A12 ensiled for 90 days. The AAS samples were analysed for nutritional composition, silage quality, and *in vitro* techniques (Hohenheim and daisy incubation) for quantification of methane and nutrient digestibility.

Results: The supplementation of acacia leaves, and molasses decreased the NH_3-N production and butyric acid contents in the silage while increased AA and DM contents of the silage. In the Hohenheim technique, M5 increased the total gas production and improved the *in vitro* dry matter digestibility, true NDF digestibility and lowered the organic matter digestibility of AAS than the control. Moreover, acacia leaves addition resulted in reducing the CH_4 production, total VFAs and ruminal NH_3-N (an indicator for N excretion) concentration.

Conclusion: The results illustrated that lower level of acacia leaves (3%) in combination with molasses (M5+A3) applied prior to ensiling alfalfa are useful to reduce silage pH, preserve the nutrients by reducing proteolysis, could improve the energy and protein utilization in animals and lowered the environmental impact of alfalfa silage and ruminant production. However, further in vitro studies are required to explore the full potential of acacia leaves with molasses inclusion in AAS.

Key words: alfalfa silage; acacia leaves; molasses; methanogenesis; proteolysis

ÖZET

Yonca Silajına Farklı Seviyelerde İlave Edilen Kurutulmuş Akasya Ağacı (*Robinia pseudoacacia*) Yaprakları ve Melasın Silaj Kalitesi İn Vitro Rumen Fermantasyonu Metan Üretimi ve Ham Besin Madde Sindirilebilirliği Üzerine Etkileri

Amaç: Son yıllarda, ruminantlar için sürdürülebilir ve alternatif yem maddelerine olan ilgi artmaktadır. Ruminantlar için yem kaynağı olarak kullanılabilecek olan Akasya, bol miktarda yaprak üreten bir baklagil ağacı olup yaprakları protein ve yoğun tanenler açısından zengin bir atık üründür. Düşük düzeyde suda çözünür karbonhidrat içermesi , yüksek tamponlama kapasitesi ve yoğun proteoliz ile besin kayıplarına eğilimli olması nedeniyle yonca bitkisinin silolanması zordur. Bu nedenle, bu çalışma da akasya ağacı yaprakları ve melas ilavesinin yonca silajının (AAS) fermantasyon kalitesi, in vitro rumen fermantasyonu, besin sindirilebilirliği, anti-metanojenik özelliği ve amonyak azotu (NH_3-N) atılımı üzerindeki etkilerini değerlendirmek için yapılmıştır. Çalışma aynı zamanda ruminant üretim sisteminde alternatif ve sürdürülebilir bir besleme kaynağı bulmayı da amaçlamaktadır.

Materyal ve Metot: Temmuz 2022'de Türkiye'nin Erzincan (39° 44′ 47″ N ve 39° 29′ 29″ E) ilinde yetiştiriciliği yapılan Yonca (*Medico sativa L.*), 3. Biçim ve orta çiçeklenme döneminde hasat edilmiştir. Akasya ağacının yaprakları (A; *Robinia pseudoacacia*), Türkiye'nin Erzurum (39° 54′ 31″ N ve 41° 16′ 37″ E) ilinde en az 10 ağaçtan olacak şekilde Haziran ayının başlarında toplanmıştır. Toplanan taze yapraklar laboratuvarda oda sıcaklığında 1 hafta boyunca havada kurutulmuş olup, şeker pancarı melası (M) (Brix değeri% 85), yerel bir şeker fabrikasından temin edilmiştir. Toplanan taze yonca, 20 mm boyutunda çim biçme makinesi ile doğranmıştır. Doğranan yonca, yığın şeklinde sekiz gruba ayrılmış olup her grup 4 paralel olarak çalışılmıştır. Gruplar; kontrol (C), %5 melas (M5), %3 akasya yaprağı (A3), %6 akasya yaprağı (A6), %12 akasya yaprağı (A12), ve akasya yaprakları ile melas kombinasyonları olan M5+A3, M5+A6, M5+A12 şeklinde her biri 1 litre hacimli cam kavanozların içerisine 700’er gr örnek konulup ağızları sıkıca kapatılarak oda sıcaklığında laboratuvarda 90 günlük siloya bırakılmıştır. Toplanan taze yonca ve akasya yaprakları ile silaj örneklerinin kuru madde içerikleri, etüvde 48 saat boyunca 65°C’de bekletilerek tespit edilmiştir. Ardından tüm örneklerin, besin bileşim analizi için Wiley değirmeni (ZM200, Retsch GmbH) ile (1 mm boyutunda) öğütülerek AOAC’nin standart yöntemleri kullanılmıştır. Nitrojen (N), eter

ekstrakt (EE) ve kül içerikleri AOAC'nin standart yöntemleri ile belirlenmiştir. Nitrojen (N), Kjeldahl yöntemi kullanılarak analiz edildi ve ardından ham protein değerini hesaplamak için 6.25xN kullanılmıştır. EE, dietil eter kullanılarak Soxhlet sisteminde belirlenmiştir. Ham lif (CF), nötr deterjan lifi (NDF) ve asit deterjan lifi (ADF), Ankom 2000 lif analizörü (Ankom Technology, Macedon NY, ABD) kullanılarak tespit edilmiştir. Hohenheim ve Daisy inkübasyon gibi *in vitro* teknikler ile sırasıyla metan ve besin sindirilebilirliğinin nicelendirilmesi için kullanılmıştır.

Bulgular: Akasya yapraklarının kimyasal kompozisyonunda %12,5 kuru madde de kondanse tanen (KT) bulunmaktadır. Bu çalışmada, kurutulmuş akasya yaprağı: %3 (KT; silajın 3.75 g/kg), %6 (KT; silajın 7.5 g/kg) ve %12 (KT; silajın 15 g/kg) şeklinde üç farklı oranda kullanılmıştır. Akasya yaprakları ile melas eklemesinin, silajdaki NH_3-N üretimini ve bütirik asit içeriğini azaltırken, silajın acetic asid (AA) ve KM içeriğini artırdığı gözlemlenmiştir. Hohenheim tekniğinde, kontrol grubuna göre M5 grubunda melasın toplam gaz üretimini artırdığı ve *in vitro* kuru madde (KM) sindirilebilirliği, gerçek NDF sindirilebilirliği ile organik madde sindirilebilirliğini iyileştirdiği tespit edilmiştir. Ayrıca, deneme gruplarına akasya yaprağı eklemenin metan üretimini, toplam UYA'ları ve ruminal NH_3-N (NH_3-N, N atımı için iyi bir gösterge) konsantrasyonunu azalttığı görülmüştür.

Kimyasal analiz sonuçlarına göre, yonca ve akasya ağacı yapraklarının besin profillerinin birbirine benzer olduğunu gözlemlenmiştir. Akasya yaprakları yoncaya göre ham protein yönünden daha yüksek fakat ham lif ve NDF içeriği olarak yoncaya göre düşük tespit edilmiştir. *In vitro* teknik ile yapılan ölçümlerde, akasya yapraklarının taze yoncaya kıyasla rumen NH_3, toplam UYA, toplam gaz ve metan üretimi sırasıyla 198.33 mg/dl, 52.63 mM/L, 23.27 ml ve 3.57 ml tespit edilmiş ve yoncadan düşük bulunmuştur. Yonca silajına (AAS) melas (M) eklenmesiyle fleig skoru ve fiziksel özellikleri iyileştirdiği gözlemlenmiştir. Yonca silajına eklenen akasya yapraklarının oranının artmasıyla silajın fleig skorunu düşürdüğü ancak, akasya yapraklarının AAS ve M ile kombinasyon yaparak birlikte silajlanması fleig skorunu iyileştirdiği tespit edilmiştir. Melas %5 (M5) katkısı ile yapılan yonca silajında pH düşerken, akasya yaprakları (A) katkısı ile AAS'da pH kuadratik olarak arttı ve en yüksek pH değeri kontrol grubuna kıyasla A6 grubunda gözlemlenmiştir. Ancak M5 ile A (%3, %6, and %12) kombinasyon grubpları kontrol grubuna göre daha düşük pH değeri elde ettiği bulunmuştur. Silajlara M5 eklenmesi AA %'sini artırdığını ancak propiyonik asit (PA) %'sini kuadratik olarak düşürmektedir. Buna ek olarak, kontrol grubuna göre bütirik asit (% BA) değerini düşürmektedir. Akasya katkılı gruplarda AA %'si kuadratik olarak azaltmış olup, değerlerin kontrol ve M5 grubuna göre A12 hariç yüksek olduğu görülmüştür. Akasya yapraklarının AAS içindeki oranının artması PA %'sini kuadratik olarak arttırmış, ancak PA % değeri kontrol grubuna göre en yüksek A3 grubunda bulunmuştur. Ancak, M5+A3 ve M5+A6 kombinasyonu, M5 grubunda daha yüksek AA% ve PA% elde edilmiştir. A6 ve A12 grubunda BA% kontrol grubundan daha düşüktür. Silajdaki melas (M5) ve akasys (A) takviyesi, BA% 'ti kontrol ve M5 grubundan daha da azaltmıştır. Yalnızca M5 ve A veya her ikisinin kombinasyonunun takviyesi, AAS'daki LA% üzerinde etkili olmadı, ancak A3 grubununda kontrolden daha düşük bir LA% seviyesi gözlemlenmiştir. AAS'nin A ve M5 kombinasyonu ile takviyesi kuru maddeyi % (KM) lineer artırırken, ruminal NH_3 konsantrasyonu lineer olarak azaltmıştır. Kuru madde sindirilebilirliği (KMS), NDF sindirilebilirliği (NDFS), organik madde sindirilebilirliği (OMS), toplam sindirilen besin madde miktarı (TDN), lifli olmayan karbonhidratlar (NCF), göreceli yem değeri (RFV), göreceli yem kalitesi (RFQ), kuru madde alımı (KMT), metabolize edilebilir enerji (ME) ve net enerji laktasyonu (NE_L) en yüksek M5 grubunda tespit edilmiştir. A6 grubu hariç AAS'deki OMS, ME ve NE_L gibi parametrelerin artması control grubuna göre önemli ölçüde azaltmıştır. Sonuç olarak, melas ve akasya yapraklarının (A) eklenmesi, deneme gruplarının sindirilebilirlik ve kalite parametreleri üzerindeki olumsuz etkisini önemli ölçüde azalttığı tespit edilmiştir. M5+A kombinasyonlarındaki melasın (M5), daha düşük seviyelerde akasya yaprakları içeren gruplarda (A3) etkili bir şekilde KMS, NDFS, OMS, TDN, NCF, RFV, RFQ, KMT, ME ve NE_L'i iyileştirdiği gözlemlenmiştir.

Bu sonuçlar, M5+A3'ün AAS kalitesini ve sindirilebilirliğini iyileştirmek için en uygun kombinasyon olduğunu göstermiştir. *In vitro* inkübasyonun 24 saati sonunda, gerçek sindirim derecesi (GSD), gerçek sindirilen besin madde miktarı (GSBM), gerçek kuru madde sindirilebilirliği (GKMS), gerçek NDF sindirilebilirliği (GNDFS), partisyon faktörü (PF), mikrobiyal protein (MP), mikrobiyal protein sentezleme etkinliği (MPSE) ve gerçek organik madde sindirilebilirliği (GOMS) değerlerinin kontrol grubuna en yüksek M5 grubunda gözlemlenmiştir. Ancak, AAS'daki akasya yaprağı (A) oranının artması tüm bu parametreleri olumsuz etkiledi ve sonuç olarak kontrol ve M5 grubundan daha düşük bulunmuştur. Bununla birlikte, melas ve akasya yapraklarının kombinasyonu ile bu parametrelerin iyileştiği gözlemlenmiştir. *In vitro* ruminal fermantasyonun 24 saati sonunda AAS gruplarının pH değerlerinin etkilenmediği ancak, AAS'daki akasya yaprağı (A) oranının artması toplam UYAs'ı ve ruminal NH_3 konsantrasyonunu lineer olarak azaltırken, toplam gaz ve metan üretimi kontrol grubuna göre kuadratik olarak azalttığı tespit edilmiştir. Dahası, kombinasyon gruplarında toplam UYAs'ta lineer bir azalmayı görülmüş olup ruminal NH_3 konsantrasyonu M5+A12'de en düşük olmak üzere kuadratik olarak azalmışır. Kontrol grubuna göre metan (%) M5+A3'te daha düşük bulunmuştur.

Sonuç: Sonuçlar, yonca silajına şeker pancarı melas ile akasya yaprağı ilavesinin silaj fermantasyon kalitesini iyileştirmede daha etkili olduğunu, rumen CH_4 üretimini ve yonca silajının NH_3-N konsantrasyonunu azalttığını göstermiştir. Bu bulgular, akasya ve melas kombinasyonunun daha verimli olduğu hakkındaki hipotezimizi kanıtlamıştır. Bununla birlikte, M %5 (M5+A3) ile kombinasyon halinde düşük seviyede akasya yaprağı (%A3) ilavesi, silajın besin içeriğini korumak için oldukça etkili olmuştur. Ayrıca M5+A3, yonca silajının besin parçalanabilirliği üzerinde herhangi bir olumsuz etki olmaksızın pH'ı, proteolizi, ruminal CH_4 üretimini ve NH_3-N konsantrasyonunu azaltmıştır. Dahası, silajın besin parçalanabilirliğini (IVDMD ve TNDFD) iyileştirmiştir. Daha düşük seviyede akasya yaprağı (KM'nin %3'ü) ve melas (KM'nin %5'i) eklenmesinin yonca silajının fermantasyon kalitesini, enerjisini ve ruminantlarda protein kullanımını iyileştirmek ve yonca silajının çevresel etkisini azaltmak için faydalı olduğu sonucuna varılmıştır. *In vitro* rumen fermantasyonu üzerinde herhangi bir olumsuz etkisi yoktur.

Silaj pH değeri, iyi kaliteli fermantasyon için önemli bir faktördür, daha düşük pH proteolitik aktiviteleri ve istenmeyen mikroorganizmaların büyümesini inhibe eder. Önceki çalışmalar, 4.5 veya daha düşük bir pH değerinin iyi bir fermantasyon kalitesine karşılık geldiğini göstermiştir. Bu çalışmada, M5 ve M5+A3, yonca silajının silolama sonrası pH değerini önemli bir şekilde azalttı. Öte yandan, akasya yaprağı oranının artması (A6 ve A12) pH değerini artırırken akasya yaprağına melas ilavesi yonca silajının pH değerini azalttı. Daha önce yapılan benzer nitelikteki çalışmalar homofermentatif laktik asit bakteri (LAB) kültüründe fermentasyon sonucunda büyük çoğunlukla LA üretildiğini, heterofermentatif laktik asit bakteri kültürü sırasında AA ve LA'nın miktarının arttığını ifade etmişlerdir. Bu çalışmada, melas ilaveli yonca silajı grupları heterofermantatif bir özelliğe sahipti. Heterofermenatif yonca silajlarında AA miktarı arttı ve bu da silajın pH'sında azalmaya neden oldu. Ayrıca bu silajlarda BA ve PA düzeyleri de azaldı. Akasya yaprağı ilaveli gruplarda AA içeriği kontrol grubundan daha yüksekken BA içeriği kontrol grubundan daha düşüktü. Ancak, silajlara akasya yaprağı ve melas karışımı ilave edildiğinde AA içeriğini önemli ölçüde artarken BA içeriği azaldı. LA içeriği tüm silaj gruplarında önemli bir değişiklik göstermedi. Bilindiği üzere, propiyonik asit (PA) üretimi çoğunlukla *Propionibacterium*, *Clostridium propionicum* ve *Selenomonas ruminantium* tarafından gerçekleştirilir. Ancak bu bakterilerin fermentasyon faaliyetleri 4.5'ten düşük pH'ya sahip ortamlarda inhibe olur. BA ise genellikle *Clostridium butyrate* tarafından üretilir ve bu bakteriler daha düşük pH koşullarında baskılanır. BA fermentasyon bozukluklarının bir göstergesi olup amino asit fermentasyonu sırasında üretilir ve besin kaybına neden olur. Silajda oluşan heterofermantatif karakterli fermentasyonun, yoncanın (KM'sinde %26.2) ve akasya yapraklarının (KM'de %30.3) yüksek protein içeriği ve eklenen melas (%5) miktarının silajın fermentasyon karakterini etkili bir şekilde homofermantasyona dönüştürememesinden kaynaklandığı düşünülmektedir. Silajın LA içeriği %5'lik melas ilavesinden etkilenmemesi bu durumu ispatlar niteliktedir.

Baklagil silajlarında, çözünür azot ve NH_3-N seviyelerinin yüksek olmasının başlıca nedeni *Clostridia sp.*'nın güçlü proteolitik aktivitesidir. Tanenlerin protein bağlayıcı özellikleri, silaj fermantasyonu sırasında NPN ve NH_3-N konsantrasyonlarının azalmasıyla ilişkilendirilmiştir. Silajda üretilen NH_3-N miktarı, silolama sürecindeki proteolitik aktiviteyle doğrudan ilişkilidir ve silajın tüketimi ile ters orantılıdır. Bu çalışmada, yonca silajına melas (%5), akasya yaprağı (tüm dozlar) ve bunların karışımın yonca silajına ilavesiyle silajın BA ve NH_3-N düzeyleri önemli ölçüde azaldı. Bu çalışmada meydana gelen düşük proteolitik aktivite (HP tüketimi) yonca silajının daha iyi korunmasından kaynaklanıyor olabileceği düşünülmektedir. Silajın fiziksel özellikleri (koku, renk ve tekstür) yonca silajına melas (%5) ilavesiyle iyileşirken yonca silajına akasya yaprağı ilavesi bu özellikleri olumsuz etkiledi. Ancak, diğer tüm uygulama gruplarındaki silajlara ait fiziksel özellikler bakımından gruplar arasında anlamlı bir fark yoktu. Tanenler, CH_4 üreten rumen mikroorganizmalarının aktivitesini azaltarak veya selüloz ve hemiselüloz gibi yapısal karbonhidratlarla stabil kompleksler oluşturarak sindirimlerini azaltırlar ve bu sayede ruminal CH_4 üretimini sınırlandırırlar. Bu çalışmada, akasya yapraklarının tek başına veya melas ile karıştırılarak yonca silajına ilave edilmesiyle CH_4 üretiminde (%) bir azalma olması, akasya yapraklarındaki KT'nin ruminal karbonhidrat fermantasyonunu inhibe ettiğini göstermektedir. Öte yandan, sonuçlar akasya yaprağı ilaveli veya melas ve akasya yaprağı karışımı ilave edilen gruplarda UYAs düzeylerinin azaldığını gösterdi. Rumen gazları, rumende karbonhidratların parçalanmasıyla üretilen uçucu yağ asitlerinden (UYAs) sentezlenir. Yapılan çalışmalarda silajlara tanen (KT & HT) ilavesi yonca silajındaki toplam UYAs konsantrasyonlarını azalttığı bulunmuştur ki bu da yaptığımız çalışmadan elde ettiğimiz bulguyu kısmen desteklemektedir. Bu çalışmada, A6 grubu veya akasya yaprağı ve melas karışımı gruplarda yonca silajına katkı maddeleri ilavesi toplam gaz üretimini artırdığı gözlendi. Ancak, %3 akasya yaprağı ilaveli gruplarda silajda üretilen gaz miktarı azaldı. M5 gruplarında CH_4 yüzdesi azaldı ve silajın toplam gaz üretimi arttı, ancak kontrol grubuna göre ruminal NH_3-N ve toplam UYAs'ı etkilenmedi. Bu çalışmada uygulama gruplarında gaz üretiminde meydana gelen artış, yonca silajındaki suda çözünür karbonhidratlar (WSC) içeriğinin artmasından kaynaklanıyor olabilir. Rumen içinde asetojenik bakteriler de mevcuttur (107 ila 108 hücre/g). Bu bakteriler şekerleri kullanarak heterotrofik veya H_2 ve CO_2'yi kullanarak ototrofik olarak büyüyebilir.

Yapılan *in vitro* araştırmalarda asetojenezin ruminal H_2 eliminasyonu için metanojeneze uygun bir alternatif olabileceğini ortaya koymuştur. Bu çalışmada, tüm uygulama gruplarında CH_4 üretiminin (%) azaldığı, AA üretiminin arttığı gözlendi. Bu bulgular ışığında silaja katkı maddesi ilavesinin (akasya yaprağı ve melas) asetojenez yolunu indüklediğini gösterebilir. *In vitro* ruminal pH, tüm uygulama gruplarında silaj pH'nın normal aralığa (5.5- 7) yakın olduğu ve bu değerin 6.95 ile 6.98 arasında değiştiğini gösterdi. Akasya yaprağının yonca silajına ilavesiyle ruminal NH_3-N konsantrasyonu azaldı, bu da protein yıkımının rumen fermantasyonu sırasında azaldığını gösterdi. Akasya yaprakları önemli KT (%12.5) kaynağıdır. Yapılan *in vitro* ve *in vivo* çalışmalarda silajlara tanen ilavesinin ruminal NH_3-N konsantrasyonunu azalttığı ifade edilmektedir. Tanenler proteinlerle kompleks oluşturarak ruminal protein yıkımını azaltabilmektedir. Yaptığımız çalışmada, kontrol grubuna göre tüm uygulama

gruplarında MP ve MPSE'nin azaldığı gözlendi. Bu azalış daha önce yapılan çalışmaların bulgularıyla çelişmektedir. Tanenin fermantasyon üzerindeki etkisi kullanılan substrata, doza ve tanen tipine bağlıdır. Bu nedenle, yaptığımız çalışmada KT içeren akasya yapraklarını bir KT kaynağı olarak kullanıldı ve sadece akasya yaprağının KT içeriği analiz edildi. Yaptığımız çalışmadan elde ettiğimiz bulguların bazıları literatürden farklıydı. Bunun temel nedeninin akasya yaprağında bulunan başka fitokimyasallar olabileceği sonucuna varıldı. Bununla birlikte, yonca silajında KT optimal dozun yanı sıra fitokimyasalların araştırılması için akasya (*Robinia pseudoacacia L.*) için daha detaylı analiz yapılması gerekmektedir. Ayrıca, yonca silajına melaslı akasya yapraklarının eklenmesinin tam potansiyelini keşfetmek için daha fazla *in vitro* çalışma yapılması önerilmektedir.

Anahtar Kelimeler: akasya yaprakları; melas; metan oluşumu; proteoliz; yonca silajı

ABBREVIATIONS LIST

ADF	:	Acid detergent fiber
CA	:	Crude ash
CH_4	:	Methane
CT	:	Condensed tannin
CO_2	:	Carbon dioxide
CP	:	Crude protein
CT	:	Condensed tannin
DM	:	Dry matter
DMI	:	Dry matter intake
EE	:	Ether extraction
GHG	:	Greenhouse gas
H_2	:	Hydrogen
IVGPT	:	*In vitro* gas production technique
IVDMD	:	*In vitro* dry matter digestibilty
Kg	:	Kilogram
ME	:	Metabolizable energy
Ml	:	Milliliter
NDF	:	Neutral detergent fiber
OMD	:	Organic matter digestibility
RFV	:	Relative feed value
RFQ	:	Relative feed quality
TDMD	:	True dry matter digestibility
TOMD	:	True organic matter digestibilty
VFA	:	Volatile fatty acids

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1- INTRODUCTION

Methanogenesis in ruminants has a significant impact on the environment due to the high global warming potential of methane. As mentioned earlier, methane is approximately 28 times more effective at trapping heat in the Earth's atmosphere than carbon dioxide, making it a potent greenhouse gas. The production of methane by ruminants is thought to be responsible for approximately 16% of global methane emissions, which contribute to climate change. Methanogenic bacteria in the rumen convert compounds such as hydrogen and carbon dioxide into methane, using an enzyme called methane monooxygenase. The process is thought to be an important aspect of the rumen ecosystem, as it helps to recycle nutrients and energy. However, the production of methane also has negative environmental impacts, as the gas contributes to climate change. Methane production in ruminants can also have an impact on the health and well-being of the animals themselves. For example, high methane production in cows has been linked to reduced feed efficiency, meaning that the animals require more feed to produce a unit of milk or meat. This can increase the cost of production for farmers and reduce the overall profitability of the operation. Additionally, high methane production has been associated with an increased risk of bloating and other digestive disorders in ruminants.

There are a lot of strategies that have been developed to reduce methane production in ruminants. These strategies can be grouped into three main categories: genetic improvement, feed management, and the use of additives.

A) Genetic improvement involves breeding animals that are more efficient at converting feed into milk or meat, and that produce less methane as a result.

B) Feed management strategies include providing ruminants with a balanced diet that is high in fiber and low in starch, as well as optimizing the timing and frequency of feed delivery.

C) The use of additives, such as certain types of bacteria or enzymes, can also help to reduce methane production in the rumen. It is worth noting that many of these strategies are still in the early stages of development, and more research is needed to fully understand their effectiveness and potential risks.

Tannins are a group of compounds that are found in a variety of plants, including trees, shrubs, and herbs. Tannins are known for their astringent taste and ability to bind proteins, and they are commonly used in the production of leather, ink, and other products. In the context of methane abatement, tannins have been shown to have potential as a feed additive for ruminant animals. Tannins have been found to have an inhibitory effect on methanogenic bacteria in the rumen, which may reduce methane production during digestion. Some studies have also suggested that tannins may improve feed efficiency in ruminants, leading to reduced methane production per unit of milk or meat produced. However, it is important to note that more research is needed to fully understand the potential of tannins as a methane abatement strategy.

2- LITERATURE REVIEW

2.1 Importance of ruminants for human population

In most developing and developed countries, animal husbandry is widely recognized as a backbone industry of the agriculture and rural economy. The livestock sector is essential for global food security, with beef consumption expected to increase by 80% by 2050 (Nadathur et al., 2017). Ruminants are the most significant part of the livestock production system because they can produce high quality protein food (milk and meat) from fibrous plants and byproducts that are indigestible to humans (Kamra et al., 2012). In fact, livestock consumed 86% of the feed that is inedible for human consumption (Mottet *et al*. 2017). Cattle produce approximately 83% of global milk (Visioli and Strata 2014), and milk output is expected to have increased by 33% and 9% in developing and developed countries, respectively, by the end of the decade (OECD/FAO). Beef accounts for 79% of the world's total meat supply (320 million tons global production volume) and ranks third among all meats consumed globally (Gerber et al., 2013).

2.2 Ruminants' impact on the environment and energy loss

The global warming potential (GWP) of greenhouse gas (GHG) such as CH_4 is 28 CO_2 equivalents (Hodnebrog et al., 2013). After CO_2, methane is the second most abundant GHG having 380 ppm concentration in the environment. The major anthropogenic sources responsible for CH_4 emissions are agriculture, natural gas distribution and landfills, and wetlands contribute most from the natural sources (Forster et al., 2007). In livestock, the main source of GHG emissions is the enteric fermentation of dairy cattle. For example, the dairy industry is responsible for 46% of the world's greenhouse gas emissions due to its annual CH_4 output of 1.1 gigatonnes (Hristov et al., 2013). However, ruminant production contributes large quantities of enteric methane (CH_4) emission leading to ecological impact. Ruminants are responsible for 16–25% CH_4

of the global GHG and about 33% of anthropogenic CH_4 emissions globally. Additionally, the CH_4 emission from ruminants represents a loss of 2% to 15% of the gross energy consumed (Eckard et al., 2010). Ruminants can produce a substantial amount of methane that accounts for 12% of their gross energy intake, which might potentially be used for physiological functions but is instead expelled into the atmosphere via eructation (Beauchemin et al., 2009). Enteric CH_4 is more potent than carbon dioxide (CO_2) in the atmosphere as well as contributes 39% to the sector's total emissions, therefore, it is attracting more attention (Gerber *et al*. 2013; IPCC 2013). CH_4 is 28 times more potent than CO_2 because it can trap more radiation and can increase the earth's temperature.

2.3 Ruminal fermentation and associated byproducts

In ruminants, the main dietary source of energy is carbohydrates. During ruminal fermentation, cellulose, hemicellulose, and starch (polysaccharides) are metabolized into glucose and other monosaccharides (mainly hexoses and pentoses). Monosaccharides undergo further metabolism to produce volatile fatty acids (VFAs), and carbon dioxide. While monosaccharides are metabolized into VFA, metabolic hydrogen ([H]) is released. In rumen hydrogen (H_2) mainly exists in two forms, dissolved H_2 (dH_2) and gaseous H_2 (gH_2) form but only dH_2 is available for ruminal microbes (Wang et al., 2014). Dihydrogen instead of accumulating in the rumen is utilized by methanogenic archaea to reduce CO_2 into CH_4 via the hydrogenotrophic pathway. Rumen can also produce methane to a lesser extent by utilizing methyl groups (methylotrophic pathway) and less commonly through acetate (Huws et al., 2018). Thus, CH_4 production represents the major sink of [H] in the fermentative process. Importantly, most [H] produced by the fermentative microbiota is transferred to methanogens as dH_2 and utilized in methanogenesis (Janssen 2010).

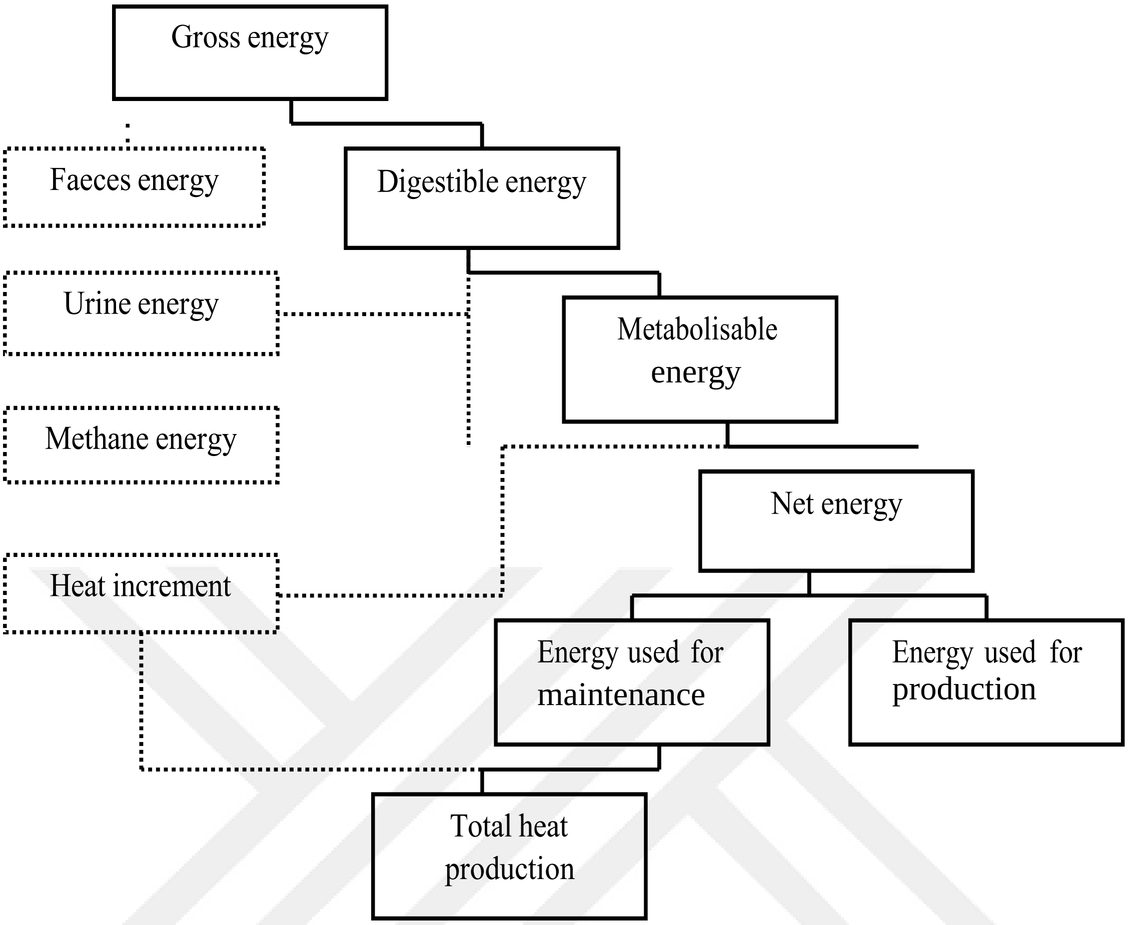

Figure 2.1 The energy turn-over in animals

2.3.1 Methanogenesis and associated microbiome in the rumen

Although numerous bacteria are capable of oxidizing NADH in conjunction with the generation of more reduced fermentation products, bacteria also rely on NADH oxidation that is triggered by the H_2 removal by other anaerobes in the rumen. For methanogenesis, archaea facilitate this by using tiny carbon-containing substrates. In the rumen, the major methanogenic reaction is (Thauer et al., 2008):

$$HCO_3^- + H^+ + 4\,H_2 \rightarrow CH_4 + 3\,H_2O$$

According to this equation, H_2 supply is essential for methanogenesis. Methanogens belong to the archaea domain from Euryarchaeota phylum and consist of five orders:

Methanosarcinales, Methanobacteriales, Methanomicrobiales, Methanopyrales and Methanococcales (Qiao et al., 2014). In a research study conducted by Henderson et al., (2015) found that there were five dominant methanogen groups that accounts for almost 90% of the fermentative archaeal communities. The dominant methanogen archaeal groups were *Methanobrevibacter gottschalkii*, *Mbb. ruminantium* (comprised 74% of all archaea) Methanosphaera sp. and two Methanomassiliicoccaceae-affiliated groups. In the rumen, about 78% of methanogens archaea were hydrogenotrophic (utilising hydrogen for methanogenesis) in nature, while 22% were methylotrophic (utilise methylgroups from methylamines or methanol), whereas acetogenic methanogens that used acetate for methane production were rare around 0.015% (Seshadri et al., 2018).

In the rumen, methanogens are present in both the fluid and solid fractions as well as adhering to the epithelium (Shin et al., 2004). Since the fractional transit rate of particles is lower than that of liquids, therefore, adhesion of ruminal microbes with particles enables them to maintain a slower growth rate for maintaining themselves in the rumen (McAllister et al., 1994). Methanobrevibacter ruminantium and Methanobrevibacter gottschalkii co-occurred with the Fibrobacteraceae and Ruminococcaceae, respectively. Adhesion to particles may be the cause of these co-occurrences (Kittelmann et al., 2013). Both bacterial families are potential cellulose degraders and release CO_2 and H_2, respectively, which are utilized by the methanogens as a substrate. In protozoal cells, methanogens can also live endosymbiotically (Fenchel and Finlay 2010). Protozoa are significant H_2 suppliers, especially for cows fed on starch-rich diets (Hegarty and Gerdes 1999). However, abundance of methanogens and protozoa may not be substantially correlated (Henderson et al., 2015).

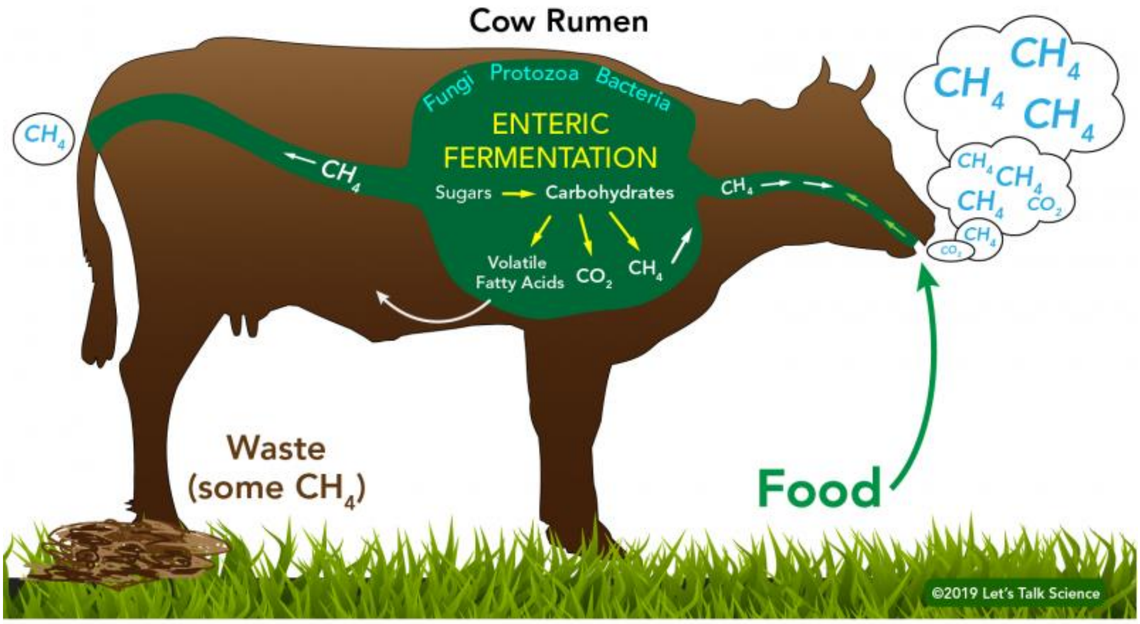

Figure 2.2 Ruminal fermentation and methane production

2.4 Tannins in ruminants feed and methane mitigation

The demand for products (beef and milk) of animal origin in vitro increase in the upcoming decades as the world's human population grows; meanwhile, the resulting environmental issues will become more prominent. Therefore, it's essential to create long-term mitigation plans for nitrogen excretion and CH_4 emissions to increase forage conversion efficiency and reduce the adverse effects of ruminant production on the environment. One such promising CH_4 slashing approach is the inclusion of tannins in forage.

The most prevalent polyphenolic contents present in plants are tannins. Tannins are divided into two types: hydrolyzable tannins (HT) and condensed tannins (CT) (Mueller-Harvey 2006). Tannins are beneficial phytochemicals that can beneficially modify ruminal microbial fermentation (Vasta et al., 2019). Additionally, tannins can modulate protein metabolism (Li et al., 2018), enteric CH_4 emission, and animal performance (Ugbogu et al., 2019), so regarded as natural ruminant feed additives.

During microbial fermentation, dietary tannins can reduce ammonia and methane production in the rumen (Carulla et al., 2005). Low levels of tannins in the feed can reduce CH_4 production and emission without negatively affecting ruminal fermentation (Jayanegara et al., 2009). While, feeding a high level of CT in the feed inhibited the terminal steps of biohydrogenation (BH) (Frutos et al., 2020). Several studies demonstrated that tannins inclusion in the diet can improve the quality of meat (Ngambu et al., 2012).

Recently, ruminant nutritionists are paying more attention to feed sources containing condensed tannins (CT) because these compounds can affect protein, fiber and lipid metabolism after interacting with ruminal microbiota (Vasta et al., 2019). In addition, CT can reduce the enteric CH_4 emissions of ruminants (Min et al., 2020). Several factors can influence the activity and efficacy of dietary tannins for CH_4 synthesis and ruminal BH, although the type and quantity of tannins play a major role (Min et al., 2020, Menci et al., 2021). Notwithstanding that methanogenic archaea utilize H_2 and CO_2 as substrates for ruminal CH_4 production (Kumar et al., 2014), there are two ways by which tannins can reduce ruminal CH_4 production: (1) inhibiting the activity of methanogenic archaea associated with CH_4 production; and (2) by forming stable complexes with carbohydrates for reducing their digestion (Min et al., 2019).

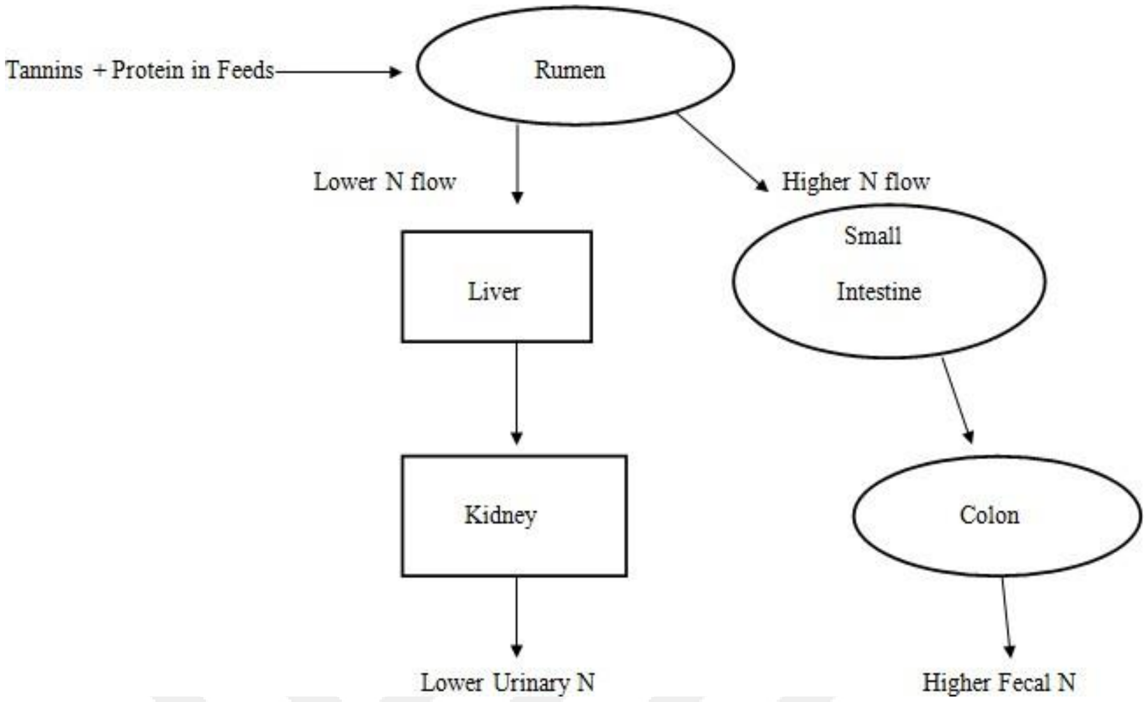

Figure 2.3. Effects of tannin on N excretion pathway

2.5 Alfalfa silage and proteolysis

Alfalfa is the main protein source that is frequently grown in enterprises and used in animal nutrition. Alfalfa is widely used in cattle feeding (Darabighane et al., 2020). Alfalfa can be given to animals as dry grass or silage can be made. Alfalfa is difficult to ensilage because it contains a low level of water-soluble carbohydrates (WSC). Alfalfa silage is prone to extensive proteolysis, and approximately 50–80% of the total N could be transformed to non-protein nitrogen (Ohshima and McDonald 1978), resulting in low protein utilization efficiency (Getachew et al., 2006), thus causing an increase in ruminal NH_3-N concentration responsible for N excretion. Different feed additives are required to reduce the environmental impact of methane emission and NH_3-N excretion from the rumen without sacrificing animal productivity.

In order to lower protein degradation during ensiling, inoculants, and organic acids are used to improve the silage fermentation, and several studies have demonstrated their beneficial impact on reducing proteolysis (Nkosi et al., 2011, Aboagye et al., 2019). Tannins are polyphenolic compounds that have the ability to bind proteins and protect them from microbial degradation (Mueller-Harvey 2006). These protein-binding characteristics of tannins suggest their potential as silage additives to limit proteolysis during ensiling (Aguerre et al., 2016, He et al., 2020). The broad-spectrum antibacterial characteristics of tannins could reduce the activity of the bacterial enzyme by precipitating and binding their cell membranes, thereby limiting the growth of harmful microorganisms (Wang et al., 2019).

2.6 Leguminous tree leaves in animal nutrition

The positive outcomes of legume forages have been known for a long period. These can be used to reduce methane emissions and change the microbial population in the rumen. In legume-based diets, energy content lost directly due to CH_4 emissions is less than the typical grass-based diets (Lee et al., 2004). Additionally, legume-based forages also encourage more feed intake and animal production (Lee et al., 2004), as well as lowering CH_4 emissions (Archimède et al., 2011). The higher dry matter intake in legume-based diets is due to chemical degradation and physical breakdown of these forages in the rumen (Dewhurst et al., 2009). When compared to conventional grass silage diets, legume-based diets tend to have a greater transit rate in the rumen due to their higher digestible energy content. Although there are many possible explanations for the higher ruminal transit rate of legumes, the most prevalent ones relate to the fiber content and particle size (Kammes and Allen 2012). Leguminous particles, which have more cuboidal-shaped pieces after ruminal breakdown, appear to move through the rumen more quickly than grass particles, which have more elongated, needle-like fragments (Kammes and Allen 2012). As undigested fibre takes longer to move through the rumen, its presence is correlated with passage rates; this, in turn, can reduce DM intake. As a result, animals benefit from a diet high in legumes in terms of DMI and milk yield, and it also helps lower intestinal methane emissions.

Recently, it has been reported that leguminous tree leaves, which are rich in protein and energy, and has grown in parks and gardens of Turkey can be used to meet the nutritional needs of ruminant animals (Canbolat 2012). Similar studies have been conducted in different countries regarding the use of leguminous trees in ruminant animal nutrition (Pal et al., 2015). In addition, it has been reported that condensed tannins in tree leaves have the potential to reduce methane production in ruminants (Jayanegara et al., 2015). However, the anti-methanogenic potentials of the leguminous tree leave grown in Turkey have not yet been demonstrated. Acacia tree leaves have a significant potential to contribute to the country's economy because it contains high nitrogen, cellulose, and polyunsaturated fatty acids content, which makes them an ideal feed source for ruminant animals (Shogren et al., 2019). In addition, is thought that its high CT contents will be effective in reducing the amount of methane produced by ruminants and the proteolysis of alfalfa silage. Denek et al. (2014) reported that the amount of tannin (174.03 g/kg DM) in acacia leaves is high.

2.7 Climate change as a future challenge and its remedies

The earth's surface temperature is rising as a result of continuous greenhouse gas emissions, particularly CH_4, which is 28% more potent than CO_2. This transition demonstrates that CH_4 emissions from ruminants into the atmosphere will be one of the greatest issues in the future. Various feed additives are being utilized to reduce the detrimental effects of methane from ruminants and to boost animal output. Condensed tannins are considered highly effective in reducing CH_4 production by reducing protein and carbohydrate degradation with their molecular binding capacity (Aboagye et al., 2018). The effectiveness of acacia leaves in feed as a rumen modifier has not been thoroughly investigated. In this context, the use of acacia leaves in the livestock industry will contribute to more economical production by preventing energy loss and creating an alternative feed raw material source. Therefore, the current project will identify the potential of acacia leaves to improve sustainable ruminant production, reduce greenhouse gas (GHG) emissions and prevent global climate changes.

2.8 Research gaps and aims of the study

In the literature, studies about the inclusion of CT from acacia tree leaves in the alfalfa silage to examine the effects on enteric methane production and ammonia-nitrogen excretion in the environment were not available. As acacia tree leaves contain high levels of condensed tannins. For this reason, the fact that the tannins in the acacia leaves have not sufficiently shown their effectiveness in reducing methane production in ruminants. In addition, the inclusion of molasses in the alfalfa silage will increase the taste and fermentation quality of the silage is one of the important points that will be emphasized in expressing the originality of the study. However, it has proven challenging to identify doses that are effective in reducing methane and have minimal detrimental effects on ruminal fermentation. In ruminants, the inconsistency of the results of various studies shows the need for more specific analyzes to check the doses of CT that are effective in reducing methane production without showing any adverse effects on ruminal fermentation.

This study aims to evaluate the effects of the combination of molasses and condensed tannins obtained from acacia tree leaves on the fermentation quality, *in vitro* gas production, nutrient digestibility, and utilization of NH_3-N of alfalfa silage. This research will help to maximize the forage conversion efficiency in ruminants by reducing gross energy losses in the form of methane and carbon dioxide. It will also reduce enteric methane production which ultimately improves the environmental conditions. It will also reduce the ammonia–nitrogen (NH_3-N) excretion in the environment to prevent air and groundwater pollution. It will help in livestock sustainability in the future which is considered a major source of human food which is now at risk due to methane and NH_3-N excretion in the environment.

3- MATERIAL AND METHODS

3.1 Hypothesis

It was hypothesized that the inclusion of different levels of acacia tree leaves containing condensed tannins in alfalfa silage can reduce the extensive proteolysis in silage, enteric methane emission, and ammonia-nitrogen excretion without any adverse effects on ruminal fermentation. However, molasses inclusion will improve the taste and fermentation quality of alfalfa silage.

3.2 Plant material collection

Alfalfa **(*Medico sativa L.*)** at 3rd cut, the mid-flowering stage were harvested from Erzincan for ensiling after dry matter analysis (28% DM) at the Department of Animal Nutrition and Nutritional diseases, Veterinary Faculty, Ataturk University. Leaves of acacia tree **(*Robinia pseudoacacia)*** were collected at the start of June from at least 10 trees from the Erzurum province of Turkey. The fresh leaves were air-dried in the laboratory and condensed tannins (CT) analysis was performed after drying. The CT concentrations in the leaves were found to be 12.5% of DM. Molasses was obtained from a sugar factory located in the Erzurum.

3.3 Experimental design for ensiling of alfalfa

The experimental design for the research is given below in table 2.1. According to the specified trial plan, the silage was ensiled in glass jars. Ensiling was done in a way that each trial group was having 4 replications and an average of 700g of silage in each jar. The alfalfa (*Medico sativa L.*) for ensiling was chopped into 20 mm dimensions, which is the ideal size for ensiling.

Table 3.1 Experimental design and treatment plan

Group	Acacia leaves DM %	Molasses DM %	Silage	Sample No.
C	0	0	Alfalfa silage	4
M5	0	5	Alfalfa silage	4
A3	3	0	Alfalfa silage	4
A6	6	0	Alfalfa silage	4
A12	12	0	Alfalfa silage	4
M5+A3	3	5	Alfalfa silage	4
M5+A6	6	5	Alfalfa silage	4
M5+A12	12	5	Alfalfa silage	4

3.4 Silage quality analysis

3.4.1 pH

The silage was opened after 90 days of ensiling and 180 ml of distilled water was added to 20 g of silage sample for pH analysis. The pH value of the filtrate obtained after filtration with cheesecloth was measured with a digital pH meter (HI 2211 PH /ORP METER) (Anonymous, 1993).

3.4.2 Fleig scoring of silage

It is stated that it is a type of scoring used to determine the quality of silage by using the dry matter and pH values of the silage obtained from the experiment (Meeske 2005). The silage fleig score was determined by using the following equation:

Fleig Score = 205 + (2 x (DM % of silage)) – (40 x pH)

Table 3.2. Alfalfa silage quality according to flieg scoring

Classification	**Score**
Excellent	81-100
Good	61-80
Medium	41-60
Low quality	21-40
Very bad quality	0-20

3.4.3. Physical analysis

Physical analysis such as odor (total score 20), color (total score 2), and structure (total score 4) of the alfalfa silage were performed by using the scale recommended by the German Agriculture Organization (DLG-1987) (Seydoşoğlu 2019).

3.4.4. Volatile fatty acids (VFAs)

The high-performance liquid chromatography (HPLC) technique was used to determine the amount of volatile fatty acids (acetic, propionic, butyric, and lactic acids) produced in alfalfa silage treated with molasses and white-flowered acacia leaves. In this method, 20 g of silage sample was added to 100 ml of distilled water and was thoroughly mixed with a grinder. Then filtration was performed through a 4-layer sterile cloth and 10ml centrifuged at 10,000 rpm for 25 minutes. After that, 5 ml filtrate was taken and placed in the vials. In the end, the readings were taken via HPLC in the DAYTAM of Ataturk University.

3.4.5. Determination of NH_3-N concentration in the silage

The legumes especially alfalfa silage undergoes microbial proteolysis during the ensiling process. In this method, 20 gr of silage sample was added to 180 ml distilled water in a beaker. After mixing with a blender for 1 minute, 100 ml filtrate was obtained by filtering through filter paper. After filtration, 100 ml of the filtrate was added to the Kjeldahl tube then 50 ml of 32% NaOH was added to it and placed in the distillation unit. While 50 ml of 2% boric acid was added to the collection chamber. After the procedure, 3-4 drops of the indicator were added and the amount of 0.1 N HCl used was placed in the formula below and the NH_3 concentration was calculated (Preston, 1986).

NH_3-N (mg/dl) = ((H *4*0.1*14)/((40*DM/100)*((CP/100)*((CP/100)/6.25))/100

H= 0.1N HCL used

CP= crude protien % of silage

DM= dry matter of silage

3.5. Chemical analysis

The AAS was analyzed by using standard methods of AOAC for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and ash contents (Vasta et al., 2019). Crude protein (N×6.25) was determined by the Kjeldahl method. The ether extract was determined using diethyl ether in a soxhlet system. Neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined according to Van Soest et al., (1991), and crude fiber (CF) were done in the Ankom 200 fiber analyzer device (Ugbogu et al., 2019). Condensed tannin contents in the leaves of acacia trees were determined by the butanol-HCl method as described by Makkar et al., (1995).

3.5.1. Determination of dry matter contents of alfalfa silage

Empty porcelain crucibles were weighed and recorded (W1). In porcelain crucibles, silage samples weighing about 1 g were weighed (W). The samples were put in porcelain crucibles and then put in the hot air oven for 24 h at 105ºC. After that, the porcelain crucibles were taken out, the samples were cooled in desiccators, and it was weighed again (W2). The samples' dry matter content was then determined as follows:

DM % = (W2 – W1/ W) x 100

W1 = porcelain crucibles weight

W2 = porcelain crucibles weight with samples after 24 h of drying

W = samples weight

3.5.2 Determination of crude protein contents of alfalfa silage

Silage samples were analyzed for crude protein with Kjeldahl method. The procedure contains 3 steps: **a)** Digestion **b)** Distillation **c)** Titration.

a) Digestion phase

Approximately 0.5-1g of silage samples were weighed and put into Kjeldahl flasks. After that, 2 catalyst tablets and 20 ml of concentrated H_2So_4 were added to each Kjeldahl flask. Then flasks were placed onto the burning chambers at 370ºC- 400ºC for 2- 3 hours. After that, the burning unit was turned off, and let flasks stay there for 5-10 minutes. The flasks were removed from the burners and placed on the Kjeldahl racks and cooled for 30 minutes.

$$\text{Organic-N} + H_2SO_4 \rightarrow CO_2 + H_2O + NH_4^{+} + SO_4$$

$$2NH_4^+ + SO_4^{-2} \rightarrow (NH_4)_2SO_4 \quad \text{(Digestion).}$$

b) Distillation phase

After wet burning, 50 ml of distilled water was added into digestion tubes to reduce the acid concentration. Afterward, the tubes were placed in the distillation device and 70 ml of sodium hydroxide solution was added. An Erlenmeyer flask was used for the distillate collection part and 10 ml of 4% boric acid and 3-4 drops of the indicator were added to it. The distillation process lasted for 3 minutes and 50 ml of distillate was collected. The color of the collected distillate was turned from red to blue due to the indicator. Then the Erlenmeyer flask was removed for titration.

$$(NH_4)_2SO_4 + 2NAOH \rightarrow Na_2SO_4 + 2NH_4OH$$

$$NH_4OH \leftrightarrow NH_3 + H_2O$$

$$NH_3 + H_3BO_3 \rightarrow NH_4H_2BO_3$$

c) Titration phase

The liquid in the Erlenmeyer flask was titrated with 0.1N HCl until the blue color of the liquid returned to a purplish-pink color. The amount of HCl used on the digital burette was recorded.

$$2(NH_4H_2BO_3) + H_2SO_4 \rightarrow 2H_3BO_3 + (NH_4)_2SO_4 \quad \text{(Titration)}$$

Crude Protein % = N x ME x 6.25 (acid ml used in titration- acid ml used in blank/W) x 100

W = Samples weight

ME = Milligram Equivalent Weight (0.014) of Nitrogen

N = Normality of the Acid (0.1 N)Used in Titration of Distillate

Figure 3.4 Distillation and combustion units used in nitrogen determination

3.5.3. Determination of NDF contents of alfalfa silage

NDF is a parameter that gives information about the volume of the feed as well as the fibrous carbohydrates in its structure e.g cellulose, hemicellulose, lignin, and heat-damaged proteins, and silicon-containing parts can be determined according to the method developed by the Van Soest et al., (1991). The solution required for NDF determination was prepared just before the analysis. NDF solution was prepared in 1800 ml of pure water by adding 120 g of FDN20C chemical, 20 ml of triethylene glycol and 4 ml of alpha-amylase dissolved at a temperature between 500 – 750 ^{0}C with a magnetic stirrer heater. The Ankom bags F57 were dried in a hot air oven for 2 h at 105 ^{0}C and weighed (W1). Approximately 0.5-1 g (W) of silage samples were weighed into the Ankom bags. After that, the Ankom bags containing silage samples were placed in an

Ankom analyzer and a 2-lit NDF solution was added. The samples were boiled for 1 hour in the Ankom analyzer. After boiling, the bags were washed with distilled water and acetone twice. The bags were dried at 80 ºC for 10-12 hours, cooled in the desiccators, and reweighed (W2). Then NDF content of the samples was determined as follows:

NDF % = (W2 – W1/ W) x 100

W1 = weight of glass crucibles

W2 = weight after drying glass crucibles with samples

W = weight of samples

3.5.4. Determination of ADF contents of silage

ADF is a parameter to determine the feed quality so if a feed contains a high level of ADF that means the feed has low energy and digestibility (Kutlu 2008). ADF solution was prepared just before starting the analysis. For this purpose, for every 2 liters of solution, 40gr of FAD20C coded chemical and 54.8 ml of concentrated sulfuric acid were added in approximately 1800 ml of distilled water and dissolved by heating at a temperature between 50ºC - 75ºC on a magnetic stirrer.

Ankom bags F57 were dried at 105 ºC for 2 hours and weighed (W1). Then approximately 0.5-1 g from the silage samples were weighed (W) into Ankom bags. After that, those Ankom bags containing silage samples were placed in an Ankom fiber analyzer and 2 lit of ADF solution were added. The samples were boiled for 1 hour in the Ankom analyzer. The bags were washed twice with distilled H_2O and acetone for 3-5 minutes. Then, bags were dried for 10-12 h at 80ºC, cooled in the desiccators, and reweighed (W2). After that, we calculated the amount of ADF in the samples as follows:

ADF % = (W2 – W1/ W) x 100

W1 = glass crucibles weight

W2 = glass crucibles weight with samples after drying

W = weight of samples

Figure 3.5 Ankom fiber analyzer to determine the NDF and ADF

3.5.5. Determination of crude fiber contents of silage

Crude fiber analysis was performed by using ANKOM 200 Fiber Analyzer. Ankom bags were numbered and tared (W1). Add 0.5 g feed sample in each and weighed (W2). After sealing the bags with heat (heat sealer) and the analyzer was set for 40 minutes. Then the cover of the device was opened and 2000 ml of 80-90°C tap water was added to it. The lid closed, and just agitate button was turned on and washed with hot water for 5 minutes. This process once again was repeated. Then 0.255 N sulphuric acid solution was added to 0.313 normality sodium hydroxide solution. Then, the cover of the device was opened and 2000 ml, 80- 90°C tap water was added. The lid was closed, agitate switch to the on position, and washed with hot water for 5 minutes. This process was repeated once again. The removed bags were kept in acetone for 3 minutes. After this process, acetone was poured and the bags were incubated at room temperature for 10 minutes. Later on, the bags were kept in the drying oven at 105°C until they reached constant weight (4 hours). The dried bags were taken from the oven into the desiccator and were kept until they reached room temperature and weighed (W3). Then, the bags were put into the tared porcelain ash crucibles and placed in the muffled furnace for burning at 550°C for 4 hours. The bags were taken from the muffle furnace to the desiccator and cooled to room temperature and weighed (W4). The CF % was calculated by using the equation given below, (Van Soest et al. 1991).

$$\text{CF \%} = ((W4 - (W1 \text{ x } C2)/\text{W2}) \text{ x } 100$$

W1 = Empty bags wight

W2 = Feed sample

W3 = Weight after extraction

W4 = Ash (OM weight)

C2 = Empty bag ash correction factor

3.5.6. Determination of ether extract contents of alfalfa silage

The samples prepared to determine the crude oil were extracted with diethyl ether and the obtained extract is reported as crude fat. Ether extract analysis was performed with a soxhlet apparatus (Figure 3.3). Cartridges were prepared with filter paper and weighed (W1) after drying in the oven. Then weighed approximately 1-2 g in 3 repetitions of feed sample (W) placed in the cartridges and the mouth of the cartridge was sealed with cotton. Sealed cartridges were placed into a drying oven at 95°C overnight. Then the cartridge was taken into the desiccator and cooled to room temperature. The cartridge was placed in the extraction part of the soxhlet device. Each extraction of the soxhlet unit of approximately 130 ml of ethyl ether so that one complete and one can half-siphon be placed in the section. The system was operated by adding ether. Extraction was completed after 8 hours. The balloons containing the cartridges were kept in the drying oven at 95^{o}C for 2-4 hours and then transferred to the desiccator. It was cooled and weighed on a precision balance again (W2). The results obtained were substituted in the formula below, and EE contents in the silage sample were determined as % (AOAC 1990).

$$EE\ \% = (W2 - W1/\ W) \times 100$$

W1 = Weight of empty cartridge

W2 = Weight of cartridge and extracted fat after drying in the oven

W = Weight of samples

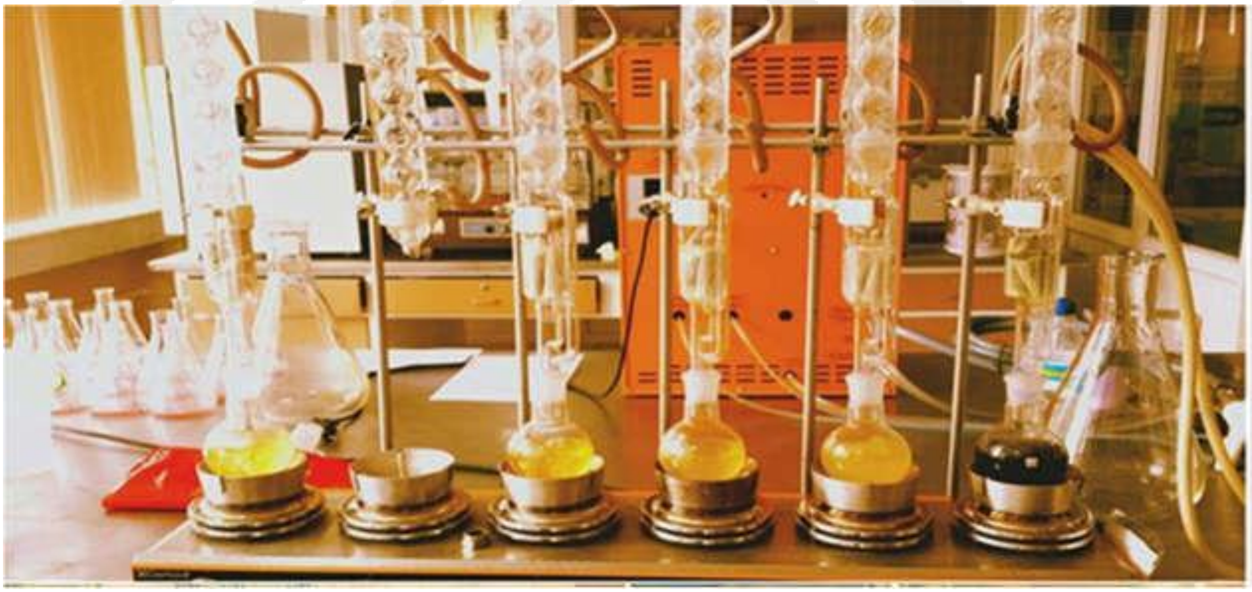

Figure 3.6. Soxhlet apparatus for ether extract analysis

3.5.7. Determination of crude ash and organic matter contents of alfalfa silage

After being burned at 550 °C in the furnace, the porcelain crucibles were cooled in desiccators and subsequently weighed (W1). The samples (1-2 g) of dried and ground silage weighed (W) in the porcelain crucibles were then burned in a blazing furnace at 550 ºC for 5-6 hours. The porcelain crucibles were taken out of the furnace and put into the desiccator for a slow cool-down. Afterward, porcelain crucibles containing samples were weighed (W2). Then, the following formula was used to calculate the crude ash content of the samples:

Crude Ash % = (W2 – W1/ W) x 100

W1 = porcelain crucibles weight

W2 = porcelain crucibles weight with samples after 5-6 h burning

W = weight of samples

And the organic matter was calculated as follows:

Organic Matter % = Dry matter % - crude ash %

3.5.8. Estimation of condensed tannins in the acacia tree leaves

Weighing about 0.01 g of tree leaves in triplicate tubes, then added 6 ml of butanol-HCl reagent **(95 mil butanol +5 ml HCl + 0.05 gram $Fe_2SO_4.7H_2O$).** The tubes were boiled for 1 hour at 100^{o}C. After cooling, the tubes were centrifuged for 10 minutes at 3000 rpm. The supernatant was collected into vials and the absorbance value was recorded at 550 nm using a CE 2030 single-beam spectrophotometer (Cecil Instruments, England). Only reagent-containing blanks were measured.

Figure 3.7. The boiling of sample tubes for tannin analysis

3.6. *In vitro* analysis of alfalfa silage

3.6.1. Rumen fluid collection

In the Hohenheim procedure, rumen fluid was taken from 2 Holstein cattle at Atatürk University Food and Livestock Application and Research Center (GHUAM). According to the literature, rumen fluid can be collected from cannulated animals (Coblentz and Walgenbach 2010, Coskun et al., 2014) or from animals slaughtered in a slaughterhouse (Dadgar 2019). In this study, rumen fluid was collected by using a catheter from donor animals, both in terms of animal welfare and for the standardization of rumen fluids to be taken. Donor animals were fed with a ration containing 60% roughage and 40% concentrate on a dry matter basis at least 2 weeks before the experiment and the rumen fluid was taken 2 hours after the morning feeding. A vacuum rumen catheter was used while a rumen fluid sample was taken from donor animals. Two 2L thermos bottles were filled with 39 °C water and preheated. The heated water discharged just before the rumen fluid collection. Using the appropriate collection procedure, at least 2000 ml of rumen fluid was collected and placed in a thermos. The rumen contents were delivered within 30 minutes to the feed analysis laboratory at the Department of Feeds and Animal Nutrition, Faculty of Agriculture, Atatürk University. After the ruminal fermentation, *In* *vitro*, organic matter digestibility, gas production, total volatile fatty acids, and NH_3-N analysis were performed.

3.6.2. *In vitro* technique for determination of gas and methane production to be formed by the treated alfalfa silage in the rumen

In vitro technique was used with 3 blanks, 3 Hohenheim grass standards, and 3 parallel alfalfa silage samples. Approximately 0.5 g of dried and ground silage sample was weighed into 100 ml glass syringes and 40 ml of artificial saliva rumen fluid was added to them. The correction factor of the Hohenheim herb standard was applied to the values revealed at the end of 24-hour fermentation, and the amount of methane in the total gas produced was measured by using an infrared methane analyzer e.g Sensor Europe GmbH, Erkrath, Germany (Goel et al., 2008).

CH_4 production (mL) = Total gas production (mL) × Percentage of methane (%).

Preparation of artificial saliva

i) Macro element solution

5.7 gr Na_2HPO_4 + 6.2 gr KH_2PO_4 + 0.6 gr $MgSO_4.7H_2O$ was dissolved in 1 liter of distilled water into a screw cap bottle and the pH of the solution was adjusted to 6.8.

ii) Trace element solution

13.2 g of $CaCl_2.2H_2O$ + 10.0 g of $MnCl_2.4H_2O$ + 1.0 g of $CoCl_2.6H_2O$ + 0.8 g of $FeCl_2.6H_2O$ was added to the screw-capped bottle respectively and dissolved in 100 ml of distilled water.

iii) Buffer solution

35 gr $NaHCO_3$ + 4 gr $(NH_4)HCO_3$ was added into the screw cap bottle, dissolved in 1 L of distilled water and the pH of the solution was adjusted to 8.1.

iv) Resazurin solution

100 mg of Rezazurin was added into a screw-cap bottle and dissolved in 100 ml of distilled water.

v) Reduction solution

285 mg of $Na_2S.7H_2O$ dissolved in 2 ml of 1 N (Normal) NaOH + 47.5 ml of purified water. It was prepared just before the rumen fluid collection and used without waiting. The preparation of the solutions explained above was mixed in the order and amount given below:

- 474 ml of distilled water
- 237 ml macromineral solution
- 0.12 ml micromineral solution
- 1.22 ml of rezazurin solution
- 237 ml buffer solution
- 37.5 ml reduction solution

Figure 3.8. *In vitro* incubation of feed samples with rumen fluid

3.6.3. Determination of the estimated metabolizable energy, net energy lactation, and organic matter digestibility of silage

The estimated metabolizable energy (ME), net energy for lactation (NE_L), and organic matter digestibility (OMD) of alfalfa silage containing acacia tree leaves were determined by using equations as suggested by (Menke 1988).

ME (MJ/kg DM) = 2.20 + 0.1357 GP + 0.057 CP + 0002859 EE

NE_L (MJ/kg DM) = 0.54+0.0909GP+0.0038CP+0.0001733EE

OMD (%) = 14.88+0.8893GP+0.448CP+0.651CA

where GP is the 24 h net gas production (ml/200 mg), CP is a crude protein (%), EE is ether extract (%) and CA is crude ash content (%).

3.6.4. Determination of *in vitro* digestion parameters

After the gas measurement in the syringes, the rumen liquid feed sample remaining in the syringe was poured into a 250 ml beaker. Later, according to Van Soest et al. (1991) prepared 75 ml NDF solution was added into the beaker and boiled on a hot plate for 1 hour. After boiling, the solution was filtered into a previously tared glass crucible with a water trumpet, left in the oven for 24 hours at 75 °C, weighed, and put in the formula, True dry matter digestibility (TDMD), microbial protein amount (MP), microbial protein synthesis efficiency (MPSE), true digested material (TD) amounts were calculated (Blümmel et al., 1997, BAŞER and KAMALAK 2020).

TDMD = Incubated dry matter (mg) – Remaining dry matter (mg)

PF_{24} = (TDMD / Gas Production)

MPS = (TDMD – (2.2 x Gas Production)

MPSE = (((TDMD – (2.2 x Gas Production))/TDMD) x 100

TD **=** (((Incubated dry matter (mg) – Remaining dry matter (mg))/ Incubated dry matter (mg)) x 100

3.6.5. Determination of NH_3-N formed in the rumen fluid

After the 24-hour ruminal fermentation in the glass syringes, 10 ml of the ruminal fluid was taken into a 50 ml beaker to determine the ammonia nitrogen contents in it. Then 4-5 drops of 98% sulfuric were added to the beaker. It was shaken by adding acid and left to stay under laboratory conditions for 2 hours. Afterward, the contents were centrifuged at 4000 rpm for 10 minutes and 2 ml was taken from the clear upper part. It was placed in the Markham steam distillation apparatus by adding 40% NaOH to it. On the other end of the assembly, a flask containing 2% boric acid solution (5 ml) was attached, and 50 ml distillate was collected. After the distillate was taken, 3 drops from 0.25 g methyl red preparation were added to it, and titration was performed with 1/70 normal H_2SO_4 and the amount of H_2SO_4 used during the titration was noted. The amount of NH_3-N was determined according to the formula below.

$$1\text{ml N/70 } H_2SO_4 = 0.2\text{mg } NH3$$

$$\mathbf{NH_3\text{-}N\ (mg/dl)} = A \times 0.2 \times 1000/B$$

A: Amount of acid used in titration (ml) - the amount of acid used in a blind trial

B: Amount of rumen fluid used

3.6.6. Determination of Total VFAs in the rumen fluid

After the 24-hour ruminal fermentation in the glass syringes, 10 ml of the ruminal fluid was taken and centrifuged at 3000 rpm for 10 minutes. After centrifugation, 2 ml of the fluid was taken from the upper part and mixed with 2 ml of solution ($MgSO_4$ + saturated 10 N solution of H_2SO_4) and placed in the Markham Steam Distillation unit. Then 50 ml of the distillate was collected in a flask and 1% phenolphthalein as an indicator (1 g phenolphthalein + 60 ml of 99% alcohol + 40 ml of distilled water) was added. After this process, titration was done with 0.002 N NaOH until permanent pink color appear with the help of a digital burette and the amount of NaOH used was recorded. The same procedures were performed for the blind trial. The total VFAs (mmol/L) of the samples were calculated as follows.

Equivalent to 1ml of N/100 NaOH = 10 moles of VFA.

Total VFAs (mmol/L) = (A-B)/2x0.01x1000

3.6.7. Determination of Relative Feed Quality of Silage

For determination of *in vitro* true digestibility of the feed DAISY incubator device was used.

i) Buffer solution A

It was prepared by dissolving 10 g of KH_2PO_4, 0.5 g of $MgSO_47H_2O$, 0.5 g of salt, 0.1 g of $CaCl_22H_2O$, and 0.5 g of urea in 1 L of distilled water.

ii) Buffer solution B

The solution was prepared by dissolving 15.0 g Na2CO3, and 1.0 g Na2S9H2O in 1 L of distilled water.

iii) Solution preparation and mixing

The temperature of the prepared buffer solutions A and B was kept at 39 °C. Then 266 ml of solution B and 1330 ml of solution A were taken and mixed in a 2-liter flask and the pH value was measured as 6.8 at 39 °C. A total of 1600 ml solution was filled into each digestion unit of the incubator, and before the digestion units are placed in the incubator, the temperature was set to 39 °C. Then, 400 ml of rumen fluid and CO_2 gas were added to each digestion unit. Rumen fluid was taken from the animals at GHUAM. After the analysis, the filter bags were washed with acetone and dried at 105°C and weighed (D1). Filter bags were dried in a drying cabinet at 65°C for 48 hours with 0.25 g feed sample and weighed (D2). The bags were placed for incubation at 39 °C for 48 hours in Ankom DAISY incubator by adding 400 mL of rumen fluid to the prepared artificial saliva, were thoroughly washed in tap water and dried at 65 °C for 48 hours and weighed (D3).

True DM digestibility =100%- [(D3-D1)/(D2-D1)x100]

The actual NDF content of the feeds was determined in the same way as the NDF determination in the filter bags Ankom fiber device and the bags were weighed (D4). Original NDF% content of feeds D6, DM content D7.

True NDF digestibility=100%- [(D4-D1)/(D6-D7)x100]

The true organic matter digestibility of the feeds was determined by taking the same filter bags washed in NDF solution, tared and placed in a porcelain crucible, and burned in a muffle furnace at 550 °C for 3-4 hours (D5). Organic matter content of feed sample DM-CA, D8.

True OM digestibility %= 100- [(D5-D2)/(D8-D7)x100]

3.7 Statistical Analysis

All the data were analyzed by one-way ANOVA using the PROC GLM procedure. The linear model to test the effect of treatments on response variables was as follows: $y_{ij} = \mu + Trt_i + e_i$, where y = response variable, μ = population mean, Trt = group, and e = residual error [N (σ, μ; 0, 1)]. The model also LSD option to attain group mean differences. Statistical significance was considered at $P \leq 0.05$. SAS (2002) SAS User's Guide: Statistics (Version 9th.). Statistical Analysis System Institute Inc., Cary, NC, USA.

4- RESULTS

4.1 Chemical composition and in vitro fermentation characteristics of dietary ingredients

The dietary ingredients used for this *in vitro* study were alfalfa and acacia tree leaves. The chemical analysis illustrated that the nutrients profile of alfalfa and acacia tree leaves was comparable to each other. However, crude protein was higher while crude fiber and NDF contents were lower in acacia leaves compared to alfalfa (Table 4.2). The *in vitro* experiment showed ruminal NH_3, total VFA, total gas and methane production 198.33 mg/dl, 52.63 mM/L, 23.27 ml, and 3.57 ml respectively that were lower in acacia leaves compared to fresh alfalfa (Table 4.3).

Table 4.2 Chemical composition of fresh alfalfa and acacia tree leaves (mean ± standard deviation, n = 3)

Parameter	**Feed**	
	Alfalfa	**Acacia leaves**
Dry Matter (DM) %	94.57±0.18	96.18±4.27
Crude Protein (CP) %	27.07±0.72	30.43±0.97
Neutral Detergent Fiber (NDF) %	57.48±2.50	42.91±2.11
Acid Detergent Fiber (ADF) %	36.86±2.16	23.71±2.78
Crude Fiber (CF) %	33.75±2.65	11.88±0.32
Ether Extract (EE) %	1.26±0.49	1.83±0.06
Crude Ash (CA) %	7.49±0.12	6.45±0.49

Table 4.3 The effect of alfalfa and acacia tree leaves on *in vitro* ruminal fermentation and methane production (mean ± standard deviation, n = 3).

Parameters	**Feed**	
	Alfalfa	**Acacia leaves**
pH	6.92±0.01	6.92±0.01
Total VFA, mmol/L	70.73±0.28	52.63±0.18
Ruminal NH_3, mg/dl	343.33±5.86	198.33±6.66
Net gas, ml	41.20±0.93	23.27±0.26
Methane, ml	7.66±0.21	3.57±0.09
Methane %	18.59±0.08	15.35±0.47

4.2 Physical characteristics and fleig scoring of silage

Table 4.4 represents the physical characteristics (color, smell and structure of forage) and fleig score of dietary ingredients. The fleig score and physical characteristics were improved with the addition of molasses (M) in alfalfa silage (AAS). Increasing the proportion of acacia leaves (AL) in AAS reduced the fleig score of silage. However, ensiling of AL and AA in combination with M improved the fleig score.

Table 4.4 Physical characteristics and fleig score of silage

Group	Color	Smell	Structure	Fleig score
C	1	11	2	19.98
M5	2	13	4	89.56
A3	1	9	3	18.76
A6	1	9	3	17.86
A12	1	10	2	29.36
M5+A3	2	12	3	92.16
M5+A6	1	10	3	81.18
M5+A12	1	10	3	84.14

4.3 Alfalfa silage fermentation characteristics

The 5% molasses (M5) supplementation in alfalfa silage (AAS) resulted in lower pH, meanwhile, acacia leaves (A) supplementation in AAS increased the pH quadratically with the highest pH value in the A6 treatment compared to the control. However, a combination of M5 with A (3,6,12) resulted in lowered pH than the control. The results depicted that M5 treatment increased the AA % (6.68 vs 3.49; $p<0.05$) meanwhile decreased the PA% quadratically and BA % linearly ($p<0.05$) than the control. In contrast, AA % was reduced quadratically within the acacia-supplemented groups but was higher ($p<0.05$) than the control and M5 group except for A12. Increasing the proportion of acacia leaves in AAS increased the PA% quadratically ($p<0.05$) than the M5 treatment but the highest PA% in the A3 treatment was observed than the control (3.51 vs 1.69).

However, the combination of M5+A3 and M5+A6 resulted in a higher AA% and PA% respectively than the M5 treatment ($p<0.05$). The BA % in treatment A6 and A12 was lower ($p<0.05$) than the control. The supplementation of M5 and A in the silage further reduced the BA% ($p<0.05$) than the control and M5 treatment. The supplementation of solely M5 and A, or a combination of both did not affect the LA% in the AAS except A3 treatment which represented a lower LA% ($p<0.05$) than the control. Linear increase ($p<0.05$) in the dry matter % (DM) while linear decrease ($p<0.05$) in the ruminal NH_3 concentration of AAS was observed with the supplementation of A, M5, or a combination of the control. The treatment M5+A12 represented the highest DM% (34.98 vs 23.29) and M5 showed the lowest ruminal NH_3 concentration (1.59 vs 7.11) than the control.

Table 4.5 Effects of ensiling alfalfa with molasses and acacia tree leaves on pH, DM%, NH_3-N and volatile fatty acids production (mean ± standard deviation, n = 3).

Group	C	M5	A3	A6	A12	M5+A3	M5+A6	M5+A12	P Value
pH	5.79±0.06^{c}	4.35±0.04^{e}	5.88±0.24b^{c}	6.13±0.14^{a}	6.05±0.08^{ab}	4.36±0.06^{e}	4.68±0.12^{d}	4.77±0.06^{cd}	0.0001
AA %	3.49±0.15^{e}	6.68±0.11^{c}	9.03±0.76^{a}	5.54±0.30^{d}	6.73±0.27^{c}	7.87±0.25^{b}	7.22±0.16^{bc}	7.41±0.04^{bc}	0.0001
PA %	1.69±0.13^{bc}	0.65±0.19^{d}	3.51±0.53^{a}	1.83±0.18^{bc}	1.79±0.27^{bc}	1.29±0.59^{bcd}	2.07±0.28^{b}	1.13±0.20^{cd}	0.0004
BA %	4.21±0.18^{a}	2.50±0.62^{bc}	5.05±0.47^{a}	2.82±0.11^{b}	1.80±0.13^{cd}	0.40±0.40^{e}	1.10±0.36^{de}	0.20±0.08^{e}	0.0001
LA %	90.61±0.29^{a}	90.17±0.34^{a}	82.4±1.68^{b}	89.82±0.44^{a}	89.69±0.61^{a}	90.44±0.91^{a}	89.61±0.22^{a}	91.27±0.27^{a}	0.0001
DM %	23.29±0.62^{e}	29.09±0.85^{d}	24.89±1.10^{e}	29.03±0.26^{d}	32.99±0.60^{ab}	30.59±0.97^{dc}	31.50±0.31^{bc}	34.98±0.61^{a}	0.0001
NH_3 -N	7.11±0.71^{a}	1.59±0.22^{c}	6.87±0.17^{a}	3.63±0.52^{b}	3.46±0.58^{b}	4.41±0.34^{b}	3.62±0.59^{b}	3.57±0.44^{b}	0.0001

AA= acetic acid, **PA**= propionic acid, **BA**= butyric acid, LA= lactic acid, **DM**= dry matter of fresh silage %, **NH_3 -N**= ammonia nitrogen concentration (mg/dl)

**Means with different letters are significantly different at $p < 0.05$

4.4 Chemical composition of alfalfa silage

Increasing the proportion of acacia leaves (A) in AAS linearly increased the DM% with the highest in A12 (90.85 vs 86.78) than the control and M5 treatment. Simultaneously, the combination of M5 and A also linearly increased the DM% more than the control ($p<0.05$). The supplementation of M5 and A didn't affect the CP% than the control. However, the combination resulted in a quadratically increase of CP contents ($p<0.05$) with the highest in M5+A12 than the control. In the M5 treatment, NDF, ADF and CF contents were lower while EE contents were higher ($p<0.05$) than the control and A-supplemented AAS. Increasing the proportion of A in AAS linearly increased the NDF and ADF contents decreased quadratically than the control and M5 treatment. Despite this, M5+A3 represented the lowest NDF (43.99 vs 47.90) and ADF contents ($p<0.05$) than the control. While CF contents were significantly lower in the M5+A12 treatment (25.14 vs 28.03) than M5 treatment. Ether extract contents increased quadratically by increasing the proportion of A in AAS, meanwhile, M5+A12 resulted in highest EE contents than the control.

Table 4.6. The chemical composition of alfalfa silage supplemented with 5% molasses (M5), acacia leaves 3% (A3) acacia leaves 6% (A6), acacia leaves 12% (A12), and a combination of acacia and molasses (M5+A3, M5+A6, and M5A+12) and control (C) after 90 days of ensiling (mean ± standard deviation, n = 3).

Group	**C**	**M5**	**A3**	**A6**	**A12**	**M5+A3**	**M5+A6**	**M5+A12**	**P Value**
DM %	86.78 ± 0.56^{e}	87.89 ± 1.06^{de}	87.91 ± 0.12^{de}	88.48 ± 0.54^{cde}	90.85 ± 0.30^{ab}	89.41 ± 1.09^{bcd}	90.13 ± 0.47^{abc}	91.57 ± 0.07^{a}	0.0009
CP %	26.22 ± 0.13^{abc}	25.57 ± 0.20^{bc}	25.45 ± 0.95^{bc}	25.01 ± 0.44^{c}	25.41 ± 0.36^{bc}	26.91 ± 0.76^{ab}	26.07 ± 0.23^{bc}	27.59 ± 0.36^{a}	0.0363
NDF %	47.90 ± 0.26^{c}	41.35 ± 0.83^{e}	52.27 ± 0.38^{b}	53.17 ± 0.95^{ab}	55.75 ± 0.40^{a}	43.99 ± 1.05^{de}	47.11 ± 1.96^{c}	46.55 ± 0.99^{cd}	0.0001
ADF %	37.34 ± 0.40^{c}	30.14 ± 0.64^{f}	40.52 ± 0.79^{a}	38.65 ± 0.28^{bc}	39.44 ± 0.53^{ab}	31.16 ± 0.61^{ef}	34.24 ± 0.72^{d}	32.35 ± 0.60^{e}	0.0001
CF %	35.85 ± 0.30^{a}	28.03 ± 0.50^{c}	31.40 ± 1.44^{b}	31.70 ± 0.44^{b}	31.96 ± 0.64^{b}	27.57 ± 0.10^{c}	28.92 ± 0.40^{c}	25.14 ± 1.27^{d}	0.0001
EE %	2.08 ± 0.06^{e}	2.72 ± 0.09^{ab}	2.39 ± 0.06^{cd}	2.12 ± 0.16^{de}	2.44 ± 0.13^{bc}	2.44 ± 0.09^{bc}	2.55 ± 0.03^{abc}	2.77 ± 0.12^{a}	0.0015

DM= dry matter %, **CP**= crude protein %, **NDF**= neutral detergent fiber %, **ADF**= acid detergent fiber, **CF**= crude fiber %, **EE**= ether extract

**Means with different letters are significantly different at $p < 0.05$

Table 4.7. The effect of alfalfa silage treated with acacia tree leaves and molasses on ruminal fermentation, *in vitro* digestibility, RFV, RFQ , and other parameters metabolizable energy and net energy (mean ± standard deviation, n = 3)

Group	C	M5	A3	A6	A12	M5+A3	M5+A6	M5+A12	P Value
DDM	59.81±0.31[d]	65.42±0,50[a]	57.34±0.61[f]	58.79±0.22[de]	58.18±0.41[ef]	64.62±0.48[ab]	62.23±0.56[c]	63.70±0.47[b]	0.0001
RFV	115.58±0.75[d]	138.08±1,88[a]	106.5±1.36[e]	108.54±1.12[e]	105.64±0.78[e]	131.15±2.64[b]	121.74±3.55[c]	125.07±1.53[c]	0.0001
RFQ	128.53±2.36[bc]	147.19±2,46[a]	124.63±1.11[c]	116.87±2.19[d]	111.69±1.10[d]	147.34±2.82[a]	132.81±3.47[b]	132.61±1.75[b]	0.0001
FA	1.08±0.06[e]	1.72±0.09[ab]	1.39±0.06[cd]	1.12±0.16[de]	1.44±0.13[bc]	1.44±0.09[bc]	1.55±0.03[abc]	1.77±0.12[a]	0.0015
NCF	18.60±0.16[bcd]	26.20±1.16[a]	15.15±0.88[de]	15.58±0.96[cde]	12.83±0.69[e]	21.08±1.95[b]	18.84±1.81[bc]	18.09±1.07[bcd]	0.0001
NDFCP	3.35±0.02[c]	2.89±0.06[e]	3.66±0.03[b]	3.72±0.07ab	3.90±0.03[a]	3.08±0.07[de]	3.30±0.14[c]	3.26±0.07[cd]	0.0001
NDFD	57.11±2.39[bcd]	66.92±1.12[a]	52.66±1.73[ef]	53.34±0.63[def]	50.69±1.79[f]	60.55±0.76[b]	58.52±0.61[bc]	55.78±0.26[cde]	0.0001
TDN	63.43±1.26[c]	66.48±0.34[c]	63.98±0.72[c]	60.35±0.75[d]	58.65±0.70[d]	69.23±0.53[a]	64.74±0.40[bc]	64.40±0.21[c]	0.0001
DMI	2.49±0.01[c]	2.72±0.03[a]	2.40±0.01[d]	2.38±0.02[d]	2.34±0.00[d]	2.62±0.04[b]	2.52±0.05[c]	2.53±0.03[c]	0.0001
OMD	38.69±0.69[e]	51.45±0.52[a]	36.63±0.26[f]	40.45±0.32[d]	38.82±0.02[e]	48.76±0.62[b]	44.51±0.12[c]	45.71±0.01[c]	0.0001
ME	7.10±0.12[e]	9.15±0.08[a]	6.74±0.03[f]	7.34±0.05[d]	7.10±0.02[e]	8.76±0.13[b]	8.23±0.01[c]	8,12±0.00[c]	0.0001
NE_L	3.95±0.08[e]	5.39±0.06[a]	3.69±0.02[f]	4.11±0.03[d]	3.94±0.01[e]	5.11±0.09[b]	4.74±0.01[c]	4.66±0.00[c]	0.0001

DDM= Dry matter digestibility(%) after 24 hrs, **RFV**= Relative feed value(%), **RFQ**= Relative feed quality(%), **FA**= Fatty acids(%) , **NDFn**= Nitrogen-free **NDF**(%), **NCF**= Non-fibrous carbohydrates(%), **NDFCP** = crude protein in NDF(%), **NDFD**= NDF digestibility (%) after 24 hrs, **TDN**= Total digestible nutrients(%), **DMI**= Dry matter intake(%), **OMD**= organic matter digestibility(%) after 24 hrs, **ME**= Metabolizable energy (MJ/Km of DM), **NE**= Net energy lactation (MJ/Kg of DM).
*Means with different letters are significantly different at p <0.05

4.5 *In vitro* ruminal fermentation and nutrient digestibility

In M5 treatment, dry matter digestibility (DDM), NDF digestibility (NDFD), organic matter digestibility (OMD), total digestible nutrients (TDN), non-fibrous carbohydrates (NCF), relative feed value (RFV), relative feed quality (RFQ), dry matter intake (DMI), metabolizable energy (ME), and net energy lactation (NE_L) were higher ($p<0.05$) than the control or solely acacia leaves (A) or combination of M5 and A supplemented alfalfa silage (AAS). Increasing the proportion of A in AAS resulted in a significant reduction of these parameters of AAS except OMD, ME, and NE_L in A6 treatment compared to the control ($p<0.05$). Interestingly, the addition of molasses in acacia leaves (A) supplemented AAS significantly lower the negative impact of A on these digestibility and quality parameters of the treated AAS ($p<0.05$). In M5+A treatments, it was observed that lower levels of acacia leaves (A3) with molasses (M5) effectively improved the DDM, NDFD, OMD, TDN, NCF, RFV, RFQ, DMI, ME, and NE_L of AAS than the control ($p<0.05$). These results depicted that M5+A3 is more potent to improve the AAS quality and digestibility (Table 4.7).

After 24 hours of *in vitro* incubation, true digestible level (TDL), true digestible rate (TDR), true dry matter digestibility (TDMD), true NDF digestibility (TNDFD) were higher; meanwhile, partioning factor (PF), microbial protein (MP), microbial protein synthesis efficiency (MPSE), true organic matter digestibility (TOMD) % of M5 treatment were lower compared to control ($p<0.05$). However, increasing the proportion of acacia leaves (A) in AAS negatively affected all these parameters and consequently were lower than the control and M5 treatment ($p<0.05$). In contrast, the combination of molasses and acacia leaves improved these parameters (Table 4.8).

Table 4.8. *In vitro* true digestibility, microbial protein, dry matter, NDF and organic matter digestibility of treated silage (mean ± standard deviation, n = 3).

Group	**C**	**M5**	**A3**	**A6**	**A12**	**M5+A3**	**M5+A6**	**M5+A12**	**P Value**
TDL	246.64±9.27[cd]	300.85±4.84[a]	232.40±7.68[d]	236.53±1.92[d]	230.55±7.16[d]	268.16±3.24[b]	263.29±2.80[b]	254.93±0.31[bc]	0.0001
PF	4.38±0.16[a]	3.32±0.08[de]	4.56±0.14[a]	3.86±0.09[bc]	4.04±0.12[b]	3.23±0.06[e]	3.51±0.04d[e]	3.56±0.02[cd]	0.0001
MP	122.70±8.19[a]	101.39±6.03[dc]	120.20±7.39[ab]	101.60±3.83[dc]	105.15±7.16[bc]	85.56±4.03[d]	98.29±2.80[dc]	97.26±1.03[dc]	0.0045
MPSE	49.66±1.85[a]	33.66±1.52[de]	51.62±1.57[a]	42.93±1.28[b]	45.50±1.74[b]	31.89±1.30[e]	37.32±0.67[cd]	38.15±0.36[c]	0.0001
TDR	56.63±2.29[bcd]	66.63±1.07[a]	52.22±1.72[ef]	52.97±0.5[def]	50.36±1.76[f]	60.29±0.78[b]	58.00±0.52[bc]	55.35±0.18[cde]	0.0001
TDMD %	45.02±1.97[cd]	59.63±0.99[a]	40.06±1.27[e]	41.50±0.59[de]	42.37±0.09[de]	52.05±1.13[b]	49.70±0.88[b]	48.59±1.69[bc]	0.0001
TNDFD %	57.11±2.39[bcd]	66.92±1.12[a]	52.66±1.73[ef]	53.34±0.63[def]	50.69±1.79[f]	60.55±0.76[b]	58.52±0.61[bc]	55.78±0.26[cde]	0.0001
TOMD %	94.98±0.01[a]	94.81±0.02[bc]	93.91±0.02[f]	94.36±0.02[e]	93.96±0.04[f]	94.77±0.04[c]	94.86±0.02[b]	94.58±0.01[d]	0.0001

TDL= true digestible level, **PF**= partioning factor, **MP**= metabolizable protein, **MPSE**= metabolizable protein synthesis efficiency, **TDR**= true digestible rate, **TDMD**= true dry matter digestibility, **TNDFD**= true neutral detergent fiber digestibility, **TOMD**= true organic matter digestibility

After 24 h of *in vitro* ruminal fermentation, pH values of treated AAS groups were not affected. However, increasing the proportion of acacia leaves (A) in AAS linearly reduced the total VFAs, and ruminal NH_3 concentrations while total gas and methane production were reduced quadratically compared to the control ($p<0.05$). Furthermore, combination treatments showed a linear decrease in total VFAs but ruminal NH_3 concentration reduced quadratically with the lowest in M5+A12 ($p<0.05$). The methane% was lower in M5+A3 compared to the control ($p<0.05$) as shown in Table 4.9.

Table 4.9. Alfalfa silage ruminal fermentation parameters pH, total VFAs, NH_3, *in vitro* total gas and methane production (mean ± standard deviation, n = 3).

Group	C	M5	A3	A6	A12	M5+A3	M5+A6	M5+A12	P Value
pH	6.96±0.02[a]	6.95±0.01[a]	6.97±0.02[a]	6.99±0.03[a]	6.97±0.01[a]	6.97±0.01[a]	6.96±0.01[a]	6.97±0.03[a]	0.9160
Total VFA (mmol/L	71.75±0.70[a]	71.47±0.32[a]	63.92±1.10[cd]	63.30±0.23[cd]	60.70±0.19[d]	67.80±0.37[b]	64.67±2.68[bc]	64.22±0.88[c]	.0001
NH_3 (mg/dL)	374±5.77[a]	353±3.79[ab]	316.33±2.40[c]	315.67±2.85[c]	299.33±11.62[c]	327±2.65[bc]	351.67±25.67[ab]	309.67±2,19[c]	0.0013
Net gas (ml)	25.05±0.83[f]	40.31±0.53[a]	22.68±0.26[g]	27.27±0.39[e]	25.34±0.00[f]	36.90±0.68[b]	33.35±0.00[c]	31.86±0.15[d]	0.0001
Methane(ml)	4.86±0.17[e]	7.51±0.06[a]	4.19±0.06[f]	4.99±0.13[e]	4.77±0.02[e]	6.76±0.03[b]	6.24±0.16[c]	5.88±0.07[d]	0.0001
Methane %	19.37±0.07[a]	18.62±0.10[b]	18.50±0.18[b]	18.30±0.22[b]	18.84±0.08[ab]	18.32±0.31[b]	18.71±0.47[ab]	18.45±0.25[b]	0.1189

Total VFA= Total volatile fatty acids, $\mathbf{NH_3}$ = ruminal ammonia

5- DISCUSSION

According to a previous study, the primary factor responsible for the silage dry matter (DM) losses was the growth of unfavorable microbes such as yeast, and mold during the ensiling process; however, the addition of molasses may reduce such losses (Jian et al., 2017). The effectiveness of the alfalfa nutrient preservation by the ensiling process was demonstrated in the current investigation by the fact that the DM contents remained stable or even slightly increased with the added amount of molasses (M). The protein contents of the raw ingredient for ensiling were conserved at the low pH level which restrict the proteolytic bacteria's activities (Van Man and Wiktorsson 2002). In the current study, alfalfa silages supplemented with M5, and a mix of acacia leaves, and molasses (M5+A3, M5+A6) showed stable CP contents which demonstrated the effectiveness of pH decline and protein contents preservation. While in only acacia leaves (A3, A6, A12) supplemented groups CP content was also stable which could be due to the high CP contents of acacia leaves themselves. However, M5+A12 showed a slight increase in the CP contents of the silage. During the ensilage process, lower pH promotes the more acid hydrolysis of plant cells (Ni et al., 2017) resulting in more NDF reduction. In M5 and M5+A3, NDF contents were lower than the control which is similar to the previous studies (Luo et al., 2021). Tannins have been shown to increase the concentration of NDF via binding to fiber (Chen et al., 2021). As acacia leaves are a rich source of condensed tannins. In this study, acacia leaves supplementation into alfalfa silage resulted in higher NDF contents than the control.

Silage pH value is a key factor for good quality fermentation, lower pH inhibits the proteolytic activities and growth of undesirable microbe (Muck and Pitt 1994). Previous studies indicated that a pH value of 4.5 or lower stands for good fermentation quality (Hashemzadeh-Cigari et al., 2014). In this study, M5 and M5+A3 effectively reduced the pH value of alfalfa after the ensiling. Meanwhile, the pH value was increased by increasing the level of acacia such as A6 and A12. However, acacia with molasses addition also reduced the pH value of the silage. The lactic acid bacteria (LAB) fermentation pattern indicated that LA was predominantly formed during homofermentation, while acetic acids (AA) and lactic acids (LA) were produced during the heterofermentation (Woolford 1975, Guo et al., 2018). In the present study, with the amount of added M5 turned the alfalfa silage into heterofermentation and increased the AA contents which resulted in pH reduction. Thus, results showed a reduction in BA and PA contents. In the acacia-supplemented groups, AA contents were higher while reduced the BA contents than the control. However, the mix of acacia and molasses significantly increased the AA contents but reduced the butyric acid (BA) contents in the silage. The LA contents were not changed in all groups of the silages. The production of propionic acid (PA), which is mostly carried out by *Propionibacterium, Clostridium propionicum* and *Selenomonas ruminantium* (Hashemzadeh-Cigari et al., 2014), is inhibited in environments with a pH less than 4.5 (Bjornsdottir et al., 2006). BA is mainly produced by *Clostridium butyrate* and is repressed in lower pH conditions. BA is an indicator of poor preservation and is produced during amino acid fermentation, which leads to nutritional losses (Szymanowska-Powałowska et al., 2014).

The heterofermentation pattern in the silage may be due to high protein contents of alfalfa (26.2% in DM) and acacia leaves (30.3% in DM) and with added amount of M5 had not effectively turned the silage into homofermentation. Thus, the LA contents of the silage were not affected by the M5. In legume silages, the major reason for high levels of soluble nitrogen and NH_3-N is strong proteolysis caused by *Clostridia* (Kung Jr et al., 2018). The protein-binding properties of tannins have been associated with a reduction in NPN and NH_3-N concentrations during silage fermentation (Cavallarin et al., 2007). The amount of NH_3-N produced in the silage is directly associated with the proteolytic activity during the ensiling process and inversely correlated with the voluntary intake of silage (Jeyanathan et al., 2014). In this study, the addition of M5, A, and a mix of M5 and A in the alfalfa silage significantly reduced the BA and NH_3-N. Meanwhile, it could be due to less proteolytic activity (CP consumption) and better preservation of the alfalfa silage. The physical characteristics (smell, color, and structure) of the silage were improved by the addition of the M5 but the acacia addition had more negatively affected these characteristics than the control. However, there was no significant difference between the physical characteristics of all the treatments than the control.

Tannins are known to limit ruminal CH_4 production either by decreasing the activity of CH_4 producing rumen microbes or by forming stable complexes with carbohydrates like cellulose or hemicellulose to reduce their digestion (Byeng Ryel Min 2019). In the current study, there was a decline in the percentage of CH_4 production with the addition of acacia leaves alone or mix with molasses than the control indicates that CT in acacia leaves inhibited the ruminal carbohydrate fermentation. Meanwhile, results depicted that VFAs proportion was decreased in acacia supplemented alone or mixed with molasses that were similar to another study. As rumen gases are produced from the breakdown of carbohydrates into volatile fatty acids (VFAs). Tannins (CT & HT) treatments in alfalfa silage were found to reduce total VFAs concentrations which partially supported this finding (Chen et al., 2021). In the present study, A6 or a mix of acacia and molasses groups increased the total gas production. However, A3-supplemented silage groups reduced the gas production from the silage. M5 supplementation had reduced the CH_4 percentage and increased the total gas production from the silage but had not affected the ruminal NH_3-N and total VFAs as compared to the control. The increase in gas production could be due to increasing the WSC contents in the alfalfa silage. In the rumen, acetogenic bacteria are also present (10^7 to 10^8 cells/g). It can grow heterotrophically by utilizing sugars or autotrophically by utilizing H_2 and CO_2 (Jeyanathan et al., 2014). *In vitro* research has also revealed that acetogenesis may be a viable alternative to methanogenesis for ruminal H_2 elimination (Morvan et al., 1996). In the current study, all the treatments have reduced the CH_4 production (%) but increased the AA production so it could be due to the acetogenesis pathway that supports our current findings as well.

In vitro ruminal pH, all the treatments illustrated that pH varied between 6.95 and 6.98 which were close to the normal range of pH (5.5- 7). In this study, all the treatment groups with acacia leaves had decreased the ruminal NH_3-N concentration which represents protein degradation was reduced during rumen fermentation. As acacia leaves are a source of CT (12.5%) in this study. Similarly, the supplementation of tannins (Getachew et al., 2008) *in vitro* and *in vivo* studies (Beauchemin et al., 2007) have also confirmed the reduction in ruminal NH_3-N concentration. The tannins can bind the protein and reduce the ruminal protein degradation. Furthermore, the present study had shown in the reduction of MP and MPSE in all the treatment groups than the control which was opposite to the previous findings. To what extent the tannin effects the fermentation depends on substrate used, dose, and type of tannins [59]. Thus, we have used acacia leaves as a substrate for CT and analyzed it for only CT there might be other phytochemicals that are diverting the current findings from the previous studies.

The amount of total VFAs was reduced by CT-containing acacia leaves treatments than the control group so the results were similar to the previous studies (Dschaak et al., 2011, Byeng Ryel Min 2019). In this study, the decrease in total VFAs resulted in lower *in vitro* dry matter digestibility (IVDMD) and true NDF digestibility (TNDFD) of acacia leaves treated silages. However, when acacia leaves were mixed with molasses (M5+A3) had improved the IVDMD and TNDFD but reduced the TOMD. Molasses supplementation in addition to supplying enough substrates for the sharp decline in the pH value, meanwhile, boosts microbial protein synthesis and improves the nutritional quality (Chen et al., 2020). In this study, with the amount of added M5, had decreased the MPSE while improving the ME, NE_L, RFV and RFQ of the silage than the control. In acacia leaves treated groups, IVDMD, TDMD, TNDFD, OMD, RFQ and RFV were lower than the control. Meanwhile, acacia with molasses mix also improved the IVDMD, TDMD, TNDFD, RFV, RFQ, ME, NE_L but reduced the TOMD than the control. In general, a mix of acacia leaves and molasses, i.e. M5 supplementation to A3, A6 and A12 (M5+A3, M5+A6 and M5+A12, respectively) resulted in greater variation from controls than the use of A3, A6 and A16 in terms of all traits taken together.

6- CONCLUSION

In the present study, results showed that the inclusion of acacia leaves with sugar beet molasses in alfalfa silage (AAS) is more potent to improve the silage fermentation quality, reduce enteric CH_4 production, and NH_3-N concentration. These findings were similar to our hypothesis about the combination of acacia and molasses is more effective as compared to use solely. However, the addition of a low level of acacia leaves (A 3%) in combination with M 5% (M5+A3) was highly effective to preserve the nutrient contents of the silage. Furthermore, M5+A3 reduced the pH, proteolysis, ruminal CH_4 production, and NH_3-N concentration without affecting the nutrient digestibility of AAS. Simultaneously, improved the IVDMD, and TNDFD of the AAS. It was concluded that adding a lower level of acacia leaves (3% of DM) and molasses (5% of DM) is beneficial to improve the alfalfa silage fermentation quality, energy, and protein utilization in ruminants and reduce the environmental impact of alfalfa silage without affecting the *in vitro* ruminal fermentation parameters. However, further *in vitro* studies are required to explore the full potential of acacia leaves with molasses inclusion in AAS.

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EK-1. ETİK KURUL ONAY FORMU

T.C.
ATATÜRK ÜNİVERSİTESİ REKTÖRLÜĞÜ
Rektörlük

Sayı : E-75296309-050.01.04-2200237195 11.08.2022
Konu : HADYEK Kararı.

VETERİNER FAKÜLTESİ DEKANLIĞINA

İlgi : 08.07.2022 tarihli ve E-36643897-000-2200211344 sayılı belge.

İlgide kayıtlı yazınız; Atatürk Üniversitesi Hayvan Deneyleri Yerel Etik Kurulumuzun 29.07.2022 tarihli ve 2022/8 sayılı Oturumunda Hayvan Deneyleri Yerel Etik Kurulu Başvuru Formu ve ekli belgeleri, gerekçe, amaç, yaklaşım ve yöntemler dikkate alınarak incelenmiş ve aşağıya çıkarılan 149 no'lu kararı ile sözkonusu yüksek lisans tez çalışmasının yürütülmesinin etik kurallarına uygun olduğuna, mevcut oy birliği ile karar verilmiş olup, çalışmanın Atatürk Üniversitesi Rektörlüğü, Gıda ve Hayvancılık Uygulama ve Araştırma Merkezi Müdürlüğünde (Deney Hayvanı Üretici/Kullanıcı/Tedarikçi Kuruluşlara Mahsus Çalışma İzni Belgeli) yürütülmesine ve taahhütname hükümlerine göre çalışmada kullanılan hayvanlara ait bilgilerin, T.C. Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğünün, Hayvanları Koruma Bilgi Sistemi (HAYBİS)'ne girilebilmesi için ekte sunulan "HADYEK Sonuç Raporu"nun Başkanlığımıza gönderilmesi hususunda;

Bilgilerinizi ve gereğini arz ederim.

TOPLANTI TARİHİ : 29.07.2022

TOPLANTI SAYISI : 2022/8

KARAR N0 149: Atatürk Üniversitesi Veteriner Fakültesi Dekanlığı, Zootekni ve Hayvan Besleme Bölümü, Hayvan Besleme ve Beslenme Hastalıkları Anabilim Dalı öğretim üyelerinden Prof.Dr.Mehmet GÜL'ün danışmanlığında, Atatürk Üniversitesi Rektörlüğü, Gıda ve Hayvancılık Uygulama ve Araştırma Merkezi Müdürlüğünde (Deney Hayvanı Üretici/Kullanıcı/Tedarikçi Kuruluşlara Mahsus Çalışma İzni Belgeli) yürütülecek olan Veteriner Hekim Mahmood Ul Hassan'ın **"Yonca Silajına Farklı Seviyelerde İlave Edilen Kurutulmuş Akasya Ağacı *(Robinia pseudoacacia)* Yaprakları ve Melasın Silaj Kalitesi, *İn Vitro* Rumen Fermantasyonu, Metan Üretimi ve Ham Besin Madde Sindirilebilirliği Üzerine Etkileri"** isimli Yüksek Lisans tez çalışması ile ilgili Veteriner Fakültesi Dekanlığının 08.07.2022 tarihli ve E-36643897-000-2200211344 sayılı yazısı ile ekleri görüşüldü.

Yapılan görüşmelerden sonra; adı geçen Yüksek Lisans tez çalışmasının yürütülmesinin etik kurallarına uygun olduğuna, taahhütname hükümleri gereğince çalışma sonucunun Başkanlığımıza bildirilmesine, mevcut oy birliği ile kabulüne; karar verildi.

Prof.Dr. Fikret ÇELEBİ
Kurul Başkanı

Ek : Sonuç Raporu. 1 Adet.

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EK-2. ETİK BİLDİRİM VE İNTİHAL BEYAN FORMU

SAĞLIK BİLİMLERİ ENSTİTÜSÜ
Graduate School of Health Sciences

ETİK BİLDİRİM VE İNTİHAL BEYAN FORMU[1]

Öğrencinin Adı ve Soyadı	Mahmood Ul Hassan
Öğrencinin Numarası	20025302005
Ana Bilim Dalı	**Hayvan Besleme ve Beslenme Hastalıkları**
Öğrencinin Kayıtlı Olduğu Program Türü	Yüksek Lisans

Yukarıda bilgileri verilen tezin intihal tespit yazılımıyla (Turnitin) yapılan tarama sonucunda elde edilen benzerlik oranları aşağıdaki gibidir. Beyan edilen bilgilerin doğru olduğunu, aksi hâlde doğacak hukuki sorumlulukları kabul ve beyan ederiz.

Bölümler	**Benzerlik Oranı**	**Maksimum Benzerlik Oranları**
I. Giriş	10%	% 15
II. Genel Bilgiler	12%	% 35
III. Materyal ve Metod	15%	% 35
IV. Bulgular	8%	% 15
V. Tartışma	9%	% 20

***Not:** Yedi kelimeye kadar benzerlikler ile Başlık, Kaynakça, İçindekiler, Teşekkür, Dizin ve Ekler kısımları tarama dışı bırakılabilir. Yukarıdaki azami benzerlik oranları yanında tek bir kaynaktan olan benzerlik oranlarının %5'den büyük olmaması gerekir.*

Tez Yazarı (Öğrenci)	**Tez Danışmanı**
Mahmood Ul Hassan	**Prof. Dr. Mehmet Gül**

[1] Bu form bilgisayar ortamında doldurulmalı, çıktısı imzalanıp Tez Savunması Jüri Öneri Formu'yla birlikte Ana Bilim Dalı Başkanlığı aracılığıyla ÜBYS üzerinden Enstitüye iletilmelidir.