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Research Article

Effect of Dried Thyme Pulp *(Tymbra Spicata L. spicata)* on Fermantation Quality and *In Vitro* Organic Matter Digestibility of Meadow Grass and Alfalfa Silages

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Abstract

The aim of the study was to evaluate the possible effect of dried Thyme *(Thymbra spicata* L.) pulp (DTP) on first-cut meadow grass *(Poa trivialis* L.) and alfalfa *(Medicago sativa* L.) silage fermantation parameters and *in vitro* organic matter digestility. Experimental silage consisted of four replicates for each silage group (Grass, (G) and Alfalfa (L), respectively). Each silages groups were supplemented with DTP at 0, 0.5, 1.0, 3.0, and 5.0% level, respectively. The silos were stored for 60 d at room temperature (about 22°C). Results showed that silages added with DTP had higher amounts of NDF, acetic acid and propionic acid whlist lower amounts of lactic acid and NH_3N-TN compared to those of control (P<0.001). DTP significantly reduced microbial load of silage opened at 60 days without changing lactobacilli (P<0.001). It was concluded that the reducing in the load of undesirable microorganisms *(Enterobacter,* yeast and mould) without causing a decline in the number of lactobacilli could be provided significantly advantages in terms of improving the aerobic stability of the silages.

Keywords: *Thyme (Tymbra Spicata L. spicata) pulp, Phenolic compunds, Silage quality, Organic matter digestibility, Meadow grass, Alfalfa*

Kuru Kekik *(Tymbra Spicata L. spicata)* Posasının Çayır ve Yonca Silajının Fermantasyon Kalitesi ve *İn Vitro* Organik Madde Sindirilebilirliğine Etkisi

Özet

Bu çalışmanın amacı, birinci biçim çayır *(Poa trivialis* L.) ve yonca *(Medicago sativa* L.) silajlarına farklı oranlarda ilave edilen kuru kekik *(Thymbra spicata* L.) posasının (KKP) silaj fermantasyon kalitesi ve *in vitro* organik madde sindirilebilirliği üzerine etkilerini belirlemekti. Silaj grupları her biri dört tekerrür olacak şekilde hazırlandı. Her iki silaj grubuna sırasıyla %0, 0.5, 1.0, 3.0, ve 5.0 düzeyinde kuru kekik posası ilave edildi. Birer kg cam kavanoz silajları 60 gün oda ısında bekletildi. Araştırma sonuçları, KKP ilaveli silajlarda NDF, asetik asit ve propiyonik asit miktarları artarken, laktik asit ve toplam azot içerisindeki amonyak azotu miktarının azaldığını gösterdi (P<0.001). Deneme silajlarında organik maddenin sindirilme derecesinde bir değişiklik tespit edilmedi. Altmışıncı günde açılan silajlarda, KKP ilavesinin laktobasil sayısında bir değişiklik oluşturmadan istenmeyen mikroorganizma yükünü önemli derecede düşürdüğü belirlendi (P<0.001). KKP'nın silajların laktobasil sayısında herhangi bir düşüşe neden olmadan istenmeyen mikroorganizmalar (Enterobakter, maya ve küf) üzerindeki bu önleyici etkisinin silajların aerobik stabilizasyonunu iyileştirmesi bakımından önemli avantaj sağlayabileceği sonucuna varıldı.

Anahtar sözcükler: *Kekik (Tymbra Spicata L. spicata), Fenolik bileşikler, Silaj kalitesi, Organik madde sindirilebilirliği, Çayır silajı, Yonca*

INTRODUCTION

Silage quliaty and the resistance to aerobic deterioration are directly related to the fermentation types and the fermentation products by microbial activity during the ensiling. Consequently, phenolics- rich ingredients such as medicinal and aromatic plants or its essential oils derived from herbs have a selective antimicrobial properties and a

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potential in binding of proteins. Therefore, these products can regulate the silage microbiology and proteolysis in silages. On the other side, Turkey exports average 8000 tones dried thyme every year [1]. Additionally, 1000 tones of thyme are consumed as condiments/culinary herbs and herbal tea and also around 1500 tones of them are used in essential oil (EOs) production within Turkey [2]. EOs have received attention in recent years as potential 'natural' alternatives for replacing antibiotic growth promoters (AGPs) in animal diets due to their positive impact on growth performance, gut microbiota, animals products and welfare [3,4]. Only about 0.5-3% essential oil containing active biological compounds *such as carvacrol, thymol, ocimene, and γ-terpinene is obtained from thyme (Thymbra spicata)* by distillation [5]. On the other hand, less amount of essential oil but higher amounts of medicine-aromatic plant water and herbal pulp were obtained during the production of plant exctraction process. Medicine-aromatic plant water contains similar active ingredients to be less than the essential oil [4]. As a by-product, approximately same amount of proceed thyme remained after exctraction process and it is defined as organic waste. Most silages materials are ensilaged at a dry matter content between 20-50%. Within this dry matter range, many enzymes in the plant are still active at ensiling [6]. Also the wide variety of bacteria, yeasts and molds can all grow within this range. So it is a substantial challenge to bring all of this biological activity under control, but that is what a well-managed ensiling process does [6]. There are two key: the creation of an anaerobic environment and the fermentation of sugars by lactic acid bacteria to lactic acid and other products are the main two processes that preserve the raw silage materils in the silo. Thyme pulp is considered to have many biological-active compounds such as polyphenols, which might be act a silage fermentation inhibitor by changing silage microbiota population and their fermentations metabolites. Unfortunately, there are no data about its nutrient composition, active- biological compounds, availability of feed additive, and natural feed preservation as silage. Therefore, in the present study, the effects of different levels of thyme *(Thymbra spicata* L.) pulp on fermantation parameters, *in vitro* organic matter digestility, and microbial load of meadow grass *(Poa trivialis* L.) and alfalfa *(Medicago sativa* L.) silages were investigated.

MATERIAL and METHODS

Silage Preparation and Treatments

Thyme pulp *(Thymbra Spicata L. spicata)* was kept at room temperature (about 22°C) till reach to constant weight for drying process (dried thyme pulp, DTP) after water distilation process of fresh material. Meadow Grass *(Bromus inermis)* and first cut at the early-bloom stage of lucerne *(Medicago sativa* L.) of fresh material, was used as silage material. The chopped fresh silage materials were weighed, and DTP was mixed by hand and placed into 1.5 L. anaerobic glass jars (silos) by hand compressing to a final density of about 800g L^{-1} on a weight basis (w/w). Both of grass (G) and alfalfa (A) silages groups were supplemented with DTP at 0, 0.5, 1.0, 3.0, and 5.0% level (ten treatments and four replicates). The silos were stored at room temperature (about 22°C) for 60 days.

Analytic Procedures

Total phenolics (gallic acid, equivalent mg kg^{-1}) amount of DTP was determined according to Bae and Suh [7] and Cuendent et al.[8], respectively. Total phenol concentration was calculated from the calibration curve formed by gallic acid and total phenols were expressed as gallic acid equivalent mg kg^{-1}. Condensed tannin contents of Thyme pulp, meadow grass and alfalfa were estimated by butanol-HCl method as suggested by Makkar et al.[9].

Silage jars were opened after 60 d of ensiling. The pH values and dry-matter contents of the silages were immediately measured. Dry-matter contents of the silages were determined by drying 20 g of the ensiled forage at 105°C for 24 h in a forced-air oven and then weighing it. A total of 25 g of fresh silage was macerated with 100 mL distilled water with a high-speed blender. The macerated silage samples were filtered through two layers of cheesecloth and then the pH values of the filtrate were measured with a laboratory pH meter (Orion, Thermo Electron Corp., Kent, WA, USA). After pH determination, 10 mL filtrate was acidified with HCl and stored at -22°C for NH_3-N analysis. The NH_3-N content was analysed according to Broderick and Kang [10] by the Kjeldahl method. Volatile fatty acids (VFA) were determined by gas chromatography with Hewlett Packard-6890 equipment (Palo Alto, CA, USA). Lactic acid was determined by high-performance liquid chromatography (HPLC). Dry matter (DM), ash, and crude protein (CP) content of samples were analyzed by the AOAC methods [11]. Neutral detergent fibre (NDF) and acid detergent fiber (ADF) contents were analysed according to methods described by Van Soest et al.[12]. Four replicates were used in order to determination *in vitro* organic matter digestibilties (IVOMD, OM%) and metabolisable energy values (ME, Mcal kg^{-1} DM) of the silages samples. For this purpose, the methods described by Menke et al.[13] was used and calculated by using the equation reported by Menke et al.[14]. In order to measure *in vitro* methane production of silages, the silage samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke et al.[13]. Methane content was determined as a percentage of 24 h the total amount of gas formed [15]. For microbial enumeration four replicate used for each silage groups. For his purpose, LAB counts were determined using de Man, Rogosa, and Sharpe agar and enterobacteria counts were obtained using violet red bile agar. Yeasts and moulds were enumerated on spread plates of yeast extract and malt extract agar (pH: 3.5, obtained using sterilised lactic acid) [16].

Statistical Analysis

The number of microbial colony-forming units (CFUs) was expressed as logarithmic (log_{10}) transformation per gram of silage. All microbiological amounts from each silage sample were subjected to log transformation prior to statistical analysis. All data were analysed by one-factor ANOVA using the general linear model procedure of SAS [17]. Differences among means were determined by Duncan's multiple comparison tests at a significance level of P<0.001 [18].

RESULTS

Total phenols and condansed tannen amounts of DTP were estimated as 14.43 mg kg^{-1} equivalent gallic acid, and 5.73 g kg^{-1} DM, respectively. Condansed tannen amounts of meadow grass and alfalfa were also determinded as 5.48 and 11.14 g kg^{-1} DM. Nutritive values, metabolisable energy and *in vitro* organic matter digestibilities of dried thyme pulp, meadow grass and alfalfa were given in *Table 1*.

Table 1. *Nutrient composition, metabolisable energy value and in vitro organic matter digestibility of dried thyme pulp, meadow grass and alfalfa (in DM)*

Item	Raw Materials		
	DTP	Meadow Grass	Alfalfa
DM, %	92.90	32.99	21.98
CP, %	11.26	14.74	19.89
ADF, %	41.89	29.62	30.80
NDF, %	51.53	56.24	40.21
ME, Mcal/kg DM	1.88	1.94	2.51
IVOMD, %OM	50.97	51.66	63.98

DM: *Dry Matter,* ***CP:*** *Crude Protein,* ***ADF:*** *Acid Detergant Fiber,* ***NDF:*** *Neutral Detergant Fiber,* ***ME:*** *Metabolizable Energy,* ***IVOMD:*** *In Vitro Organic Matter Digestibility*

Compared with the control group (GS_{CTL}), increasing addition levels of DTP did not changed the DM, ADF, ME, IVOMD and CH_4 values of silages (P>0.001). Crude protein level was decreased while NDF level was increased in grass silages, especially in the addition of DTP at 5% level (P<0.001) *(Table 2)*.

When silage fermantation parameters and microbial counts of GS were investigated it was determined that adding of different levels of DTP were not changed the silage pH (P>0.001) *(Table 3)* but reduced NH_3-N/TN levels of GS especially adding level at over 3% (P<0.001). However, acetic and propionic acid levels of GS increased while lactic acid and butyric acid levels were decreased depending on the addition DTP levels (P<0.001). Increasing DTP level was shown a strong antimicrobial effect on both of aerobic (enterobacter and clostridia) and anaerobic (yeast and mould) microorganism of silages except to lactobacilli (P<0.001) *(Table 3)*. Generally, antimicrobial effect of DTP was more pronounced in the groups with dried thyme pulp at over 1%.

Effect of DTP on nutrients composition, IVOMD and ME value of alfalfa silages was presented in *Table 4*. Compared with the control group (AS_{CTL}), DM content of alfalfa silages increased by the addition of DTP at 3 and 5% levels (P<0.001) while ADF and NDF contents of those silages increased at all adding levels of DTP in alfalfa silages for 60 days (P<0.001). CP, ME, IVOMD and CH_4 values of alfalfa silages were found to be similar (P>0.001).

Compared with the control group (AS_{CTL}), the addition of DTP did not affect the silage pH (P>0.001) but reduced the NH_3-N/TN values of alfalfa silages, especially the groups with DTP at 3 and 5% levels (P<0.001) *(Table 5)*. A decrease of lactic acid levels and an increase of propionic acid levels were observed in alfalfa silages depending on the addition of TP at 1.0, 3.0 and 5.0% levels (P<0.001). All levels of DTP increased the acetic acid levels of alfalfa silages (P<0.001). Butyric acid was not detected in the alfalfa silages with DTP.

Table 2. *Effect of dried thyme pulp on nutrients composition, metabolisable energy (ME) , methane (CH_4) production and in vitro organic matter digestibility (IVOMD) of meadow grass silage*

Item	Treatments					SEM	P
	GS_{CTL}	G-DTP_1	G-DTP_2	G-DTP_3	G-DTP_4		
DM, %	32.39	31.85	32.20	32.19	33.02	0.263	NS
CP, %	14.01^a	13.14^{ab}	13.31^{ab}	12.85^{ab}	12.67^b	0.142	***
ADF, %	29.23	29.43	29.23	31.05	31.10	0.287	NS
NDF, %	48.27^b	48.56^{ab}	50.82^{ab}	51.61^{ab}	52.66^a	0.512	***
ME, Mcal/kg DM	1.91	1.90	1.91	1.90	1.95	0.046	NS
CH_4 , %	9.11	8.71	10.54	10.63	10.20	0.234	NS
IVOMD, %OM	51.93	51.18	51.58	51.50	52.19	0.278	NS

^{a,b,c} *The groups in the same line labeled different letters are statistically significant (P<0.001)* ***GS_{CTL}:*** *Grass silage with not additive,* ***G-DTP_1:*** *Grass with dried thyme pulp at 0.5%,* ***G-DTP_2:*** *Grass with dried thyme pulp at 1.0%,* ***G-DTP_3:*** *Grass with dried thyme pulp at 3.0%,* ***G-DTP_4:*** *Grass with dried thyme pulp at 5.0%,* ***DM:*** *Dry Matter, CP: Crude Protein,* ***ADF:*** *Acid Detergant Fiber,* ***NDF:*** *Neutral Detergant Fiber,* ***ME:*** *Metabolizable Energy,* ***IVOMD:*** *In Vitro Organic Matter Digestibility,* ***CH_4:*** *Amount of methane in total amount of produced gas,* ***NS:*** *Non-significant, *** P<0.001*

***Table 3.** Effect of dried thyme pulp on fermentation parameters and microbial load of meadow grass silage*

Item	Treatments					SEM	P
	GS_{CTL}	G-DTP$_1$	G-DTP$_2$	G-DTP$_3$	G-DTP$_4$		
pH	4.62	4.51	4.59	4.52	4.34	0.041	NS
NH_3-N/TN, %	9.32^a	7.89^b	7.27^{bc}	6.47^c	4.64^d	0.421	***
LA, g/kg DM	25.20^a	22.46^a	21.91^a	20.41^{ab}	16.97^b	0.771	***
AA, g/kg DM	7.54^c	13.98^b	14.61^b	15.73^b	21.11^a	1.184	***
PA, g/kg DM	0.84^b	1.14^b	2.14^b	10.29^a	13.82^a	1.486	***
BA, g/kg DM	5.29^a	3.96^a	3.74^a	4.52^a	0.35^b	0.470	***
Microbial counts, log cfu/g							
Enterobacter	120.00^a	46.67^b	8.67^c	7.67^c	6.67^c	11.86	***
Clostridia	373.33^a	300.00^a	300.00^a	123.33^b	116.67^b	29.06	***
Lactobacilli	6.03	6.10	6.20	6.24	6.28	0.283	NS
Yeast	600.0^a	466.67^a	40.00^b	16.67^b	12.30^b	71.05	***
Mould	1150^a	550^b	330^c	47^d	44^d	109.88	***

*^{a,b,c} The groups in the same line labeled different letters are statistically significant (P<0.001) **GS_{CTL}:** Grass silage with not additive, **G-DTP$_1$:** Grass with added at dried thyme pulp 0.5%, **G-DTP$_2$:** Grass with dried thyme pulp at 1.0%, **G-DTP$_3$:** Grass with dried thyme pulp at 3.0%, **G-DTP$_4$:** Grass with dried thyme pulp at 5.0%, **NH_3-N/TN:** Ammonia percentage in total nitrogen, **LA:** Lactic Acid, **AA:** Acetic Acid, **PA:** Propionic Acid, **BA:** Butyric Acid, **NS:** Non-significant, *** P<0.001*

***Table 4.** Effect of dried thyme pulp on nutrients composition, metabolisable energy (ME), methane (CH_4) production and in vitro organic matter digestibility (IVOMD) of alfalfa silages*

Item	Treatments					SEM	P
	AS_{CTL}	A-DTP$_1$	A-DTP$_2$	A-DTP$_3$	A-DTP$_4$		
DM, %	21.11^b	21.38^b	21.45^b	23.03^a	23.47^a	0.270	***
CP, %	18.77	18.50	17.67	17.60	17.45	0.162	NS
ADF, %	31.01^b	35.58^a	35.86^a	35.99^a	36.53^a	0.558	***
NDF, %	37.97^b	41.58^a	41.04^a	42.56^a	42.55^a	0.472	***
ME, Mcal/kg DM	2.48	2.50	2.42	2.40	2.39	0.081	NS
CH_4, %	11.82	11.44	11.54	11.37	11.03	0.087	NS
IVOMD, %OM	64.71	64.45	62.88	62.54	61.57	0.509	NS

*^{a,b,c} The groups in the same line labeled different letters are statistically significant (P<0.001) **AS_{CTL}:** Alfalfa silage with not additive, **A-DTP$_1$:** Alfalfa with dried thyme pulp at 0.5%, **A-DTP$_2$:** Alfalfa with dried thyme pulp at 1.0%, **A-DTP$_3$:** Alfalfa with dried thyme pulp at 3.0%, **A-DTP$_4$:** Alfalfa with dried thyme pulp at 5.0%, **DM:** Dry Matter, **CP:** Crude Protein, **ADF:** Acid Detergant Fiber, **NDF:** Neutral Detergant Fiber, **ME:** Metabolizable Energy, **IVOMD:** In Vitro Organic Matter Digestibility, **CH_4:** Amount of methane in total amount of produced gas, **NS:** Non-significant, *** P<0.001*

Microbial load of alfalfa silages were lower than compared with the grass silage. In similar to grass silage, a superior antimicrobial activity was also determined in alfalfa silage (P<0.001). Compared with control silage (AS_{CTL}) lactobacilli counts were not changed in the alfalfa silage with DTP at 0.5, 1.0, 3.0% level (P>0.001) respectively, but increased in the alfalfa silage with DTP at 5.0% level compared to that of other groups (P<0.001).

DISCUSSION

In the present study, possibility use of DTP as a silage additive in grass and alfalfa silage was investigated. When considering the nutritional proterties of DTP, it can be said that DTP has the potential to be used as a silage additive *(Table 1*). Also, DTP may have silage additive potantial due to its phenolic compounds which could reduce protein degradation and growth undesirable microorganisms (enterobacters, yeasts and moulds) in silages. Addition of DTP did not changed DM, ADF, IVOMD, ME and CH_4 level whlist the addition decreased CP level and increased NDF values of meadow grass silages (P<0.001) *(Table 2)*. There are limited data on silage quality and fermantation parameters of essential oil or purified compounds derived from essential oil, such as carvacrol, eugenol, linalool, cinnamic aldehyde and thymol. In a recent study where the effect of *oregano* and *cinnamon* essential oils at 400 mg/kg level on fermentation quality and aerobic stability of field pea silage were investigated it was reported that both additives reduced dry matter losses and prevented the cell wall materials of silages [19]. In the present study, DTP addition increased the ADF and NDF levels of both

***Table 5.** Effect of dried Thyme pulp on fermantation parameters and microbial counts of alfalfa silages*

Item	Treatments					SEM	P
	AS_{CTL}	A-DTP_1	A-DTP_2	A-DTP_3	A-DTP_4		
pH	4.82	4.78	4.76	4.74	4.72	0.012	NS
NH_3-N/TN, %	20.21^a	19.65^a	19.10^a	17.37^b	16.12^b	0.415	***
LA, g/kg DM	59.85^a	50.37^a	35.58^b	30.45^b	25.98^b	3.492	***
AA, g/kg DM	15.24^d	17.44^d	24.22^c	36.30^b	42.69^a	2.876	***
PA, g/kg DM	1.31^c	1.69^{bc}	2.61^{ab}	2.87^{ab}	3.82^a	0.248	***
BA, g/kg DM	5.43^a	0.00^b	0.00^b	0.00^b	0.00^b	0.581	***
Microbial counts, log cfu/g							
Enterobacter	12.0^a	7.0^b	7.0^b	7.0^b	6.0^b	0.63	***
Clostridia	325^a	285^a	80^b	75^b	85^b	30.52	***
Lactobacilli	6.31^b	6.36^{ab}	6.41^{ab}	6.47^{ab}	6.58^a	0.271	***
Yeast	160.0^a	53.3^b	13.3^b	8.3^b	7,67^b	16.10	***
Mould	226^a	110^b	85^b	38^c	27^c	19.26	***

*^{a,b,c} The groups in the same line labeled different letters are statistically significant (P<0.001) **AS_{CTL}:** Alfalfa silage with not additive, **AS-DTP_1:** Alfalfa with added at dried thyme pulp 0.5%, **A-DTP_2:** Alfalfa with dried thyme pulp at 1.0%, **A-DTP_3:** Alfalfa with dried thyme pulp at 3.0%, **A-DTP_4:** Alfalfa with dried thyme pulp at 5.0%, **NH_3-N/TN:** Ammonia percentage in total nitrogen, **LA:** Lactic Acid, **AA:** Acetic Acid, **PA:** Propionic Acid, **BA:** Butyric Acid, **NS:** Non-significant, *** P<0.001*

silage samples (P<0.001). The possible mechanism of DTP on ADF and NDF values of silages could be attributed to the limited fermantation caused by the active antimicrobial compound (phenylpropanoids and hydrocarbons) of DTP. According to end-products of the expression/ metabolization of phenolic compounds by Lactic Acid Bacteria (LAB), some of them *(L. plantarum)* are activated while some others are inhibited *(Lactobacillus collinoides* and *L. brevis)* during the ensilage. Thus, a heterofermentatif process are shaped in silages [20]. These finding were in agreement with the results of Soycan Önenç et al.[19]. Therewithal, ADF (41.89%) and NDF (51.53%) values of DTP could be also contributed to those values of silage samples. A decreasing observed in DM content of alfalfa silage with DTP at 3 and 5% level compared with L_{CTL} are supported to this review. In both silage groups, the addition of DTP did not change silage pH but decreased NH_3-N/TN, lactic acid and butyric acid levels (P<0.001). Additionally, treatments increased acetic acid and propionic acid levels of both silages (P<0.001). This current silage fermantation profile indicated that interaction between the phenolic compound with lactic acid bacteria has established a heterofermentative fermentation. This fermentation type was especially pronounced in the silages with higher level of DTP (P<0.001).

These findings were in agreement with the results of the previous studies where the effects of silage inhibitors on silage quality were investigated [21-23]. In both silage groups, pH values was over 4.0 (pH: 4.34-4.62 for grass silages and 4.72-4.82 for alfalfa silages, respectively) and the addition of DTP into the silages did not change silage pH (P>0.001). As considering DM content of grass silages (31.85-33.02%), it was thought that this high level of DM caused to a prevention the decreasing of grass silage pH. For alfalfa silages, this results could be attributed to the high level of nitrogen and buffer capasity of alfalfa. Hence, previously studies reported that lower pH values was observed in the grass silages *(Perennium ryegrass)* with lower DM (26.4-28.0%) content [21] and the alfalfa silages with the addition of soluble carbohydrates (Honey Locust Pods at 20-100 g kg^{-1} fresh material) [24]. These results of previously studies are supported to this interpretation. On the other hand, high acetic acid level of silages could be also caused the current pH range, which is a weaker acid than lactic acid [24]. Additionally, purified compounds derived from essential oils such as carvacrol and tyhmol directly increased the silaj pH value [19]. Soycan-Önenç et al.[19] reported that the addition of *Origanum onites* essential at 400 mg kg^{-1} fresh pea forage, did not change the silage pH (4.40) while the addition of *cinnamon* essential at same level increased directly the silage pH (4.47) compared with the control (pH: 4.31) (P<0.001). This finding was in agreement with the results orserved from the current study. NH_3-N/TN values of both silages groups reduced by the addition of DTP, especially alfalfa silages with DTP at 3 and 5% level (P<0.001) *(Table 3* and *Table 5)*. A reducing of NH_3-N/TN values could be attributed to the limited fermantantion caused by the antimicrobial active compounds preventing the proteolysis such as polyphenols. Polyphenol compunds of DTP could be reduced nitrogen losses by binding proteins of material. Thus, in a previos study where the effects of honey locust pods (HLP) as carbohydrate source with rich polyphenols on the fermentation parameters and microbiological characteristics of alfalfa *(Medicago sativa* L.) silages were investigated, it was reported that increasing additive level of honey locust pods significantly reduced silages NH_3-N levels due to its high polyphenols compunds [24]. In contrary to the report that phenolic compounds reduce the methanogenesis, in the present study, the addition of DTP did not change methane production in both of silage groups. This could be attributed to the metabolization of phenolic compund to end-products during ensilage, and inefficacy of these end-products on inhibiting of the methanogenesis [25]. In genarally, the addition of DTP suppressed the lactic acid levels of silages and contrary to expectation, LA levels of alfalfa silages were higher than that of the grass silage (P<0.001). As DM contents of silages were considered, DM contents of alfalfa silages were lower compared with the grass silages. At this DM content, alfalfa silages might have higher water soluble carbohydrate caused to increase LA production compared with the grass silages. Also, low amount of LA in grass silage could be attribited the meadow grass used in the study that partially mixed with the legumes [26]. Hence, lactobacilli counts of alfalfa silages were higher than that of grass silages *(Table 3* and *Table 5)*. There was a reducing or a tendency reducing in LA levels of silages. This reducing was pronounced in the alfalfa silages starting from the addition of DTP at 1% level (P<0.001). This finding was in agreement with the results of Soyvan-Önenç et al.[19]. On the other hand, this reduction in LA level could be attributed to the pH range which might be contributed to acetobacter activity. LA is transformed to acetic acid by acetobacters activity at certain pH values (around pH: 4.0-4.5) causing an increasing of silage pH [25]. LA levels of a quality silages are reported as 50-70 g kg^{-1} of silage DM [23].

In the current study, pH values and LA amounts of silage were lower than generally acceptation for a quality silage. But there are different opinions on this issue. Thus, Baytok et al.[22] reported that higher pH level and lower LA amount did not adversly effect silage fermentation and it nutritive values. On the other hand, the high levels of LA are reduced the microbial protein production in ruminants, of which fed mainly silage [26]. Thereby, the higher LA level might be desirable in terms of fermentation quality but not be possible to say same thing in terms of its effect on animal performance. Maximun protection of dry matter and cell wall components of silage are essential. The source of LA in silage is either cell wall components or water soluble carbohyrdrates of the plant material. AA level of silages is also important as much as LA level in this protection [25]. Furthermore, the source of AA is LA produced in silage that is transforming to AA by asetobacter in certain pH of silage. Therefore, it can be speculated that the ideal silage fermantation should provide the minumin level of LA that will be form a protective effect in sufficient quantity. In the present study, it was observed that the addition of DTP enabled the acetobacter fermentation and thus increased the AA level by reducing the pH drop (P<0.001) *(Table 3* and *Table 5)*. The high level of AA caused by the high buffering capacity in the silages with low dry matter than 25% was the expected fermantation pattern from alfalfa silages [29]. In additionally, the silage pH values (around pH: 4.3-4.8) might be stimulated production of acetic acid. Acetic acid bacteria are obligate aerobic and acid-tolerant bacteria. When the silage pH is around 4.0-4.5, LA are transformed to AA by acetic acid bacteria for maintaining their bacterial activities [25]. Similarly, it was determined that propionic acid levels were also increased in the silage samples with added DTP (P<0.001) *(Table 3* and *Table 5)*. This could be attribured to the silage pH which allows the activity of propionic acid bacteria [25]. Acetic acid is a fungicidal agent such as propionic acid and it inhibits the growth of yeast and mold fungi [30]. There is a positive correlation among silage anerobic stabilisation and silage organic acid level, and acetic acid obviously acts as an inhibitor of the growth of spoilage organisms [25,30]. Acetic acid shows a stronger antibacterial property compared with lactic acid, especially in certain pH values (4.0-4.5), and improves aerobic stability of silages after opening [25,31]. Furthermore, it was reported that silage should be promoted by acetic acid fermantation to improve aerobic stability and acetic acid is not a factor that directly reduces the feed intake of animals [25,31]. In a previos study where the effects of various compounds on the aerobic stability of silages were evaluated, it was determined that acetic acid has been proven to be the sole substance responsible for the increased aerobic stability, and acetic acid acts as an inhibitor of spoilage organisms. This results in the higher antimicrobial activity of acetic acid in surroundings where the pH values are low (around pH 4), since a greater proportion of the acetate is not dissociated [25]. The amount of acetic acid in silages necessary to successfully preserve the silages after the silos are opened can be quite high. The silage used in an experiment required a concentration of acetic acid of more than 50 g kg^{-1} for a stability of 100 h. and lactic acid exhibited clearly lower inhibition of the yeasts and mold tested [25]. Similar results were reported by Moon [32] who observed that for the same degree of inhibition, lactate concentrations had to be about two times higher than the concentrations of acetate. Therefore, the results in the current study reconfirm the importance of undissociated acids in spoilage inhibition. Clostridia and enterobacteria are considered undesirable microorganism for silage fermentation and quality as well as yeast and mold [33-35]. Microbial load of grass silages were higher than compared with the alfalfa silage *(Table 3* and *Table 5)*. This could be related to the initial contamination of fresh plant material with soil or manure [35,36]. Additionally, the addition of DTP showed a superior antimicrabial effect and significantly reduced microbial counts, especially yeast and mould *(Table 3* and *Table 5)* (P<0.001). Unfortunately, we could not determine the antimicrobial potential of DTP and aerobic stabilities of silage samples. Therefore, it could not be possible to reveal the relationship among these parameters in the concrete. But, it could be argued that both phenolic compounds [19,37] and organic acids of silage (acetic acid and propionic acid, especially in around pH: 4.0-4.5) [25] acted synergistically, thus the silage anerobic stabilities were probably improved. As far as we aware, there is no data on demonstrating the effect of phenolic-rich ingredients (feed-stuff obtained after essential oil distilation process of herbs) on silage quality. The results revealed that the ensiling of meadow grass and alfalfa by addition of dried thyme pulp increased silage fermentative quality but did not affect organic matter digestibility. Dried thyme pulp, showed antimicrobial effect by increasing acetic and propionic acid levels of both silage samples. A reducing in the load of undesirable microorganisms (yeast and mold etc.) without causing a decline in the number of lactobacilli can be provided significantly advantages in terms of improving the aerobic stability of the silages. More research is required to determine how such phenolics-rich ingredients affects the silage aerobic stability.

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