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Research Article

Use of Farmatan as an Additive to Make Alfalfa Silage

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Abstract

In this study, we investigated the effects of tannin extract of chestnut (Farmatan) obtained from sweet chestnut on alfalfa silage. Alfalfa harvested at the flowering stage was chopped to 1-2 cm in size and laid on a flat surface. Farmatan at the levels of 0 (control group), 1.5, 3.0 and 4.5% was uniformly spread by hand on the silage material. After thoroughly mixing the silage material, it was placed in 1.5-2.0-kg airtight plastic bins and stored for two months. Farmatan had significant effects on the composition of silage including dry matter (DM), crude ash, ammonia, and pH. Addition of 4.5% Farmatan reduced the ammonia content by 70.11% compared with control. Addition of 3.0% Farmatan also reduced pH by 23.8% compared with control group. The DM contents of treatment groups were changed between 18.70 and 26.57%. The highest DM content of alfalfa silage was obtained with addition of 4.5% Farmatan, whereas the lowest was found in control group. The effects of Farmatan on gas production kinetics, metabolizable energy, and organic matter digestibility were statistically significant. Addition of Farmatan at the rate of 4.5% to fresh alfalfa material can be recommended to improve the silage quality.

Key Words: Alfalfa silage, farmatan, ammonia, organic acid, silage pH.

Farmatan Silaj Katkı Maddesinin Yonca Silajı Yapımında Kullanımı

Özet

Bu çalışmada, tatlı kestane ekstraktından elde edilen silaj katkı maddesinin (Farmatan) yonca silajına etkisi araştırılmıştır. Çiçeklenme döneminde hasat edilen yonca 1-2 cm boyutunda doğranıp düz bir zemin üzerine serildikten sonra Farmatan silaj katkı maddesi %0 (kontrol), %1.5, %3.0 ve %4.5 oranlarında silaj materyali üzerine elle yayılmıştır. Daha sonra silaj materyali homojen bir şekilde karıştırıldıktan sonra 1.5-2.0 kg kapasiteli plastik silolara hava kalmayacak şekilde doldurulmuş ve 2 ay süreyle bekletilmiştir. Farmatan, silajların kompozisyonlarının kuru maddesi, ham kül, amonyak ve pH parametreleri üzerine önemli derecede etki etmiştir. Kontrol grubuyla karşılaştırıldığında, %4.5 oranında Farmatan ilavesi yonca silajının amonyak içeriğini %70.11 oranında düşürmüştür. Kontrol grubuyla karşılaştırıldığında, %3.0 oranında Farmatan ilavesi yonca silajının pH'sını %23.8 oranında düşürmüştür. Muamelelerin KM içerikleri %18.70 - %26.57 arasında değişmiş olup en yüksek kuru madde oranı % 4.5 oranında Farmatan katılan yonca silajında elde edilmiştir. En düşük kuru madde içeriğine ise kontrol grubunda rastlanmıştır. Katkı maddesi olarak kullanılan Farmatan'ın bir birim artması; silaj kuru madde içeriğinde yaklaşık olarak 1.78 birimlik artışa neden olmuştur. Sonuç olarak, yonca silajı kalitesini iyileştirilmesi için taze yonca materyaline %4.5 oranında Farmatan katılması tavsiye edilebilir.

Anahtar kelimeler: Yonca silajı, farmatan, amonyak, organik asit, silaj pH'sı.

Introduction

Silage production is a practice aimed at solving the forage scarcity problem in ruminant nutrition; hence its great importance. In Turkey, silage made from plants of the families Poaceae and Leguminosae such as clover, vetch, barley, wheat, and particularly corn, are produced either using a single plant or blending them together. Green feed materials obtained from Poaceae plants are ensiled more easily than those from Leguminosae plants, and the silage obtained from the former has higher quality.

A major problem in ensiling Leguminosae species is the fact that plants of this family do not have adequate water-soluble carbohydrates and their buffering capacity is high (Raques and Smith, 1966; Pitt 1990; McAllister et al., 1998; Davies et al., 1998; Owens et al., 1999; Altinok et al., 2005).

Because the Leguminosae do not have sufficient water-soluble carbohydrates, lactic acid bacteria cannot produce sufficient lactic acid in the ensiling process; this is the acid causing the silage pH to decline. Since it is difficult to expeditiously raise the silage pH to the desired level (pH: 4), undesirable events take place inside the feed material. As a result of these events, the silage quality considerably decreases and animals do not feel like consuming the produced silage.

Proteolysis is the most important of such events that take place because it is not able to decrease the silage pH to the desired level at the desired speed. In other words, it is the degradation of real proteins available in the feed into ammonia. As is known, proteolysis is induced during ensilage by plant enzymes and the enzymes secreted by microorganisms available on the feed material. The amount of real proteins in the silo feed decreases while the amount of ammonia increases. Silage formed by intensive proteolysis has a low efficiency of consumption by animals, and it is considerably difficult to store the available nitrogen mainly in ammonia form within the body. The largest part of the nitrogen inside the silage material that had been subject to intensive proteolysis is most of the time excreted through the urine, since it cannot be transformed into microbial protein by the microorganisms in the rumen due to lack of energy. This, in turn, results in waste of real proteins and leads to increased expenses on bypass proteins to be added to the diet. Moreover, the nitrogen excreted via urination is an important threat to the environment (Winters et al., 2000).

To obtain high-quality silage, various additives and methods are used during ensilage depending on the ensiled plant. The main objective of all these methods is to safely produce high-quality silage. Studies on silage have shown that the operations and additives applied to improve silage quality have been successful in increasing the silage quality; however, some problems were to be solved in cases in which these methods were not sufficient. Additionally, the fact that several countries have banned the use of some silage additives for being carcinogenic suggests that there is a need to find alternative and natural silage additives that are not harmful to the environment, humans, and animals (Slottner and Bertilsson 2006).

Some studies on feed including tannin have brought a new direction to research on silage. Tannin is a phenolic compound available mainly in the leaves of trees or plants such as Sainfoin and trefoil. It combines with proteins in the rumen, preventing them from over-degradation, and helps more bypass protein to proceed into the small intestine. In this way, the animal can use the proteins available in the feed more efficiently. In recent years, extracts with a high tannin content have started to be used as silage additive for the benefit of its proteins-binding property. It has thus become possible to prevent proteins from excess degradation during ensilage, and positive results are obtained in terms of animal production.

Lavrancic and Levard (2006) investigated the effects of tannins on grass silage composition and reported that addition of tannins to the fresh grass material did not substantially change the DM, ether extract, and NDF contents of silages. The crude fiber content decreased while the level of nitrogen-free extracts increased. The CP content decreased from 133 in control silage to 116 and 117 g/kg DM in silages prepared with 15 and 30 g of tannins.

Compared with other silage materials, alfalfa silage is higher in crude protein (CP), calcium (Ca), and phosphorus (P) contents, but lower in total digestible nutrients. This silage contains, on average, 17.1% CP, 1.64% Ca, and 0.26% P (Anonymous, 1981). Jatkauskas et al. (2015) studied alfalfa silage and found dry matter (DM), CP, acid detergent fiber, and neutral detergent fiber (NDF) contents of 35.54%, 22.2%, 32.6%, and 37.6%, respectively, and a pH content of 6.27.

This study was conducted to determine silage composition, the degree of *in vitro* organic matter digestion, and the impact of the latter factor on the metabolic energy content of Farmatan (chestnut tannin extract), used as an additive in alfalfa silage production.

Material and Methods

In this study, alfalfa plants harvested in the flowering period were cut down to 1-2 cm dimensions and then spread on a flat ground. Later, Farmatan (containing 75% hydrolyzed tannin, 18% sugar, 1% inorganic salt, and 6% water; pH 3.5-3.7) was dispersed homogeneously onto the silage material at the rates of 0.0%, 1.5%, 3.0%, and 4.5%. After the silage material was thoroughly blended, it was packed in airtight plastic silos with 1.5-2 kg capacity and stored for two months.

Analyses of DM, crude ash, OM, CP, acid detergent fiber (ADF), and NDF were conducted in accordance with AOAC (1990).

Twenty grams (20 g) of wet silage material were put in a blender, 180 mL distilled water were added thereafter, and the material was blended thoroughly. Later, the contents were filtered through a gauze folded 3-4 times to remove solid parts, and the pH of the filtrate was measured. Subsequently, adopting Kjeldahl method and performing distillation and titration operations for protein specification, 100 mL of this filtrate were used to determine the ammonia content (AOAC 1990).

Feed samples (0.200 g) with 30 mL solution (10 mL rumen fluid + 20 mL artificial saliva) were left for incubation at 39 ºC for 96 h inside 100-mL injectors (Menke and Steingass, 1988). Injectors were shaken for 30 min after the initiation of incubation and then within the first 10 h of incubation they were shaken every hour on the hour. Gas measurements were made after 0, 3, 6, 12, 24, 48, 72, and 96 h. Net total gas productions were calculated by subtracting gas values obtained from the blank test. All gas measurements were repeated three times. Later, using the obtained gas measurements, the amount of gas produced and the parameters related to gas production were calculated using the $y = a + b\ (1-exp^{-ct})$ model developed by Orskov and McDonald (1979), in which a = amount of gas obtained from the easily fermented part; b = amount of gas obtained from the slowly fermented part; c = b fraction fermentation rate; and t = time.

Using 24-h gas measurement levels obtained as a result of gas production and feed contents, the amount of metabolizable energy (ME) was calculated by the formula below (Menke et al., 1979) (1).

$$\text{Metabolizable energy (MJ/kg DM)} = 2.20 + 0.136GP + 0.057CP, \quad (1)$$

in which GP = 24-h gas production (mL); and CP = crude protein.

Using 24-h gas measurement levels obtained as a result of gas production and feed contents, the organic matter digestibility was calculated using the formula below (Menke et al., 1979) (2).

$$\text{OMD (\%)} = 14.88 + 0.889GP + 0.45CP + 0.0651CA, \quad (2)$$

in which OMD = organic matter digestibility; GP = gas production; CP = crude protein; and CA = crude ash content.

Unidirectional variance analysis (ANOVA) was used to determine the impact of Farmatan on silage composition and *in vitro* gas production, whereas the General Linear Model (GLM) was used to analyze the OMD and ME data. Tukey's test was applied to compare differences between means (Statistica, 1993).

Results and Discussions

Farmatan significantly affected the silage composition (Table 1). The DM contents of the silages ranged between 18.70% and 26.57% (Figure 1).

Neutral detergent fiber contents ranged between 36.08% and 44.47%, whereas the ADF contents ranged between 35.69% and 38.73%. The NDF, ADF, and ammonia contents of the silages significantly decreased with the increasing levels of Farmatan addition (Table 1).

One unit of increase in Farmatan caused the ammonia content of the silage to decrease by approximately 9.43 units (Figure 2).

Table 1. The effect of Farmatan on chemical composition and quality of the alfalfa silage

Chemical composition	Treatments					
	Control	% 1.5	% 3	% 4.5	SE	P
Dry matter (%)	18.70^{c}	22.06^{b}	25.21^{a}	26.57^{a}	0.903	***
Protein (%)	18.04^{b}	17.13^{c}	19.41^{a}	19.08^{a}	0.208	***
NDF (%)	44.47^{a}	43.36^{a}	37.74^{b}	36.08^{c}	0.441	***
ADF (%)	38.73^{a}	37.22^{ab}	35.69^{b}	36.13^{ab}	0.828	*
Ash (%)	8.80^{a}	8.31^{b}	6.97^{c}	7.12^{c}	0.150	***
Ammonia (mg/dl)	60.86^{a}	38.74^{b}	25.30^{bc}	18.19^{c}	3.36	***
pH	6.30^{a}	5.88^{ab}	4.48^{c}	5.20^{bc}	0.304	**

^{a,b,c} : Differences between means indicated by different letters in the same row are important. *: P<0.05, **: P<0.01, ***: P<0.001, SE: Mean standard of error, NDF: Neutral detergent fiber, ADF: Acid detergent fiber.

Throughout the 60-day ensilage period, the pH of the silages ranged between 4.48 and 6.30. Significant decreases (P<0.01) occurred in the silage pH with the increasing Farmatan rates. A one-unit increase in Farmatan rate resulted in a silage pH decline of approximately 0.31 units (Figure 3).

When the impact of Farmatan on the organic acid content of alfalfa silage was analyzed, we observed that Farmatan only affected isobutyric acid. However, it did not influence other organic acids such as lactic, acetic, propionic, butyric, or isovaleric acids. The isobutyric acid content increased significantly (P<0.05) with the increasing Farmatan rates (Table 2).

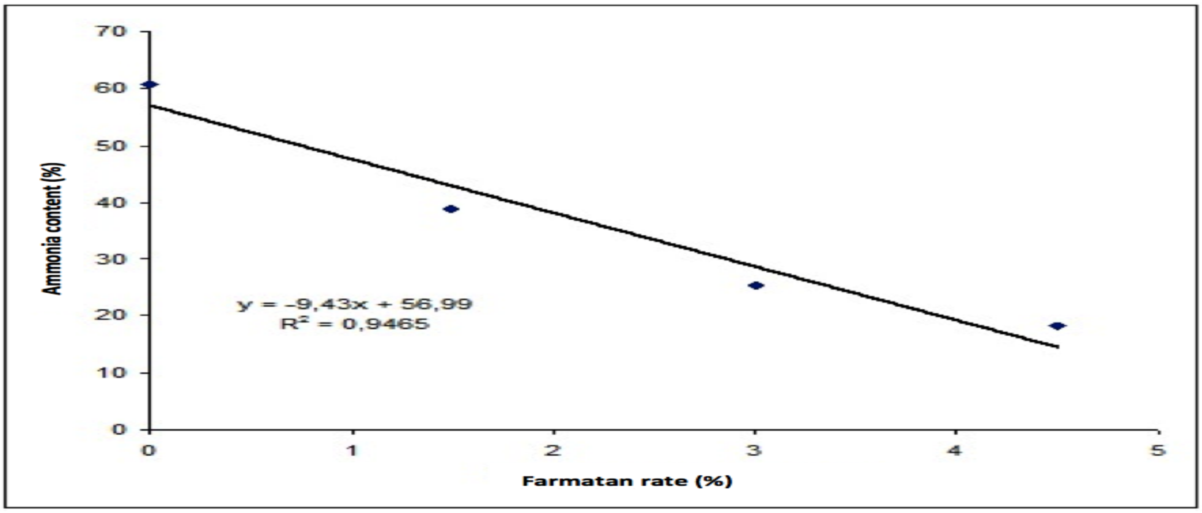

Figure 2. The relation between Farmatan rate and ammonia content.

Table 2. Farmatan's impact on organic acid content of alfalfa silage

Organic acids	Control	% 1.5	% 3.0	% 4.5	SE	P
Lactic acid	0.10	1.12	1.16	1.10	0.81	NS
Acetic acid	0.92	0.95	0.66	0.75	0.26	NS
Propionic acid	0.36	0.11	0.00	0.09	0.13	NS
Isobutyric acid	0.59^{b}	1.81^{ab}	2.73^{a}	2.30^{ab}	0.57	*
Butyric acid	0.84	0.26	0.00	0.09	0.26	NS
Isovaleric acid	0.14	0.18	0.04	0.05	0.10	NS

^{abc} Differences between means indicated by different letters in the same row are important. SE: Standard error, NS: Non significant, *: P>0.05.

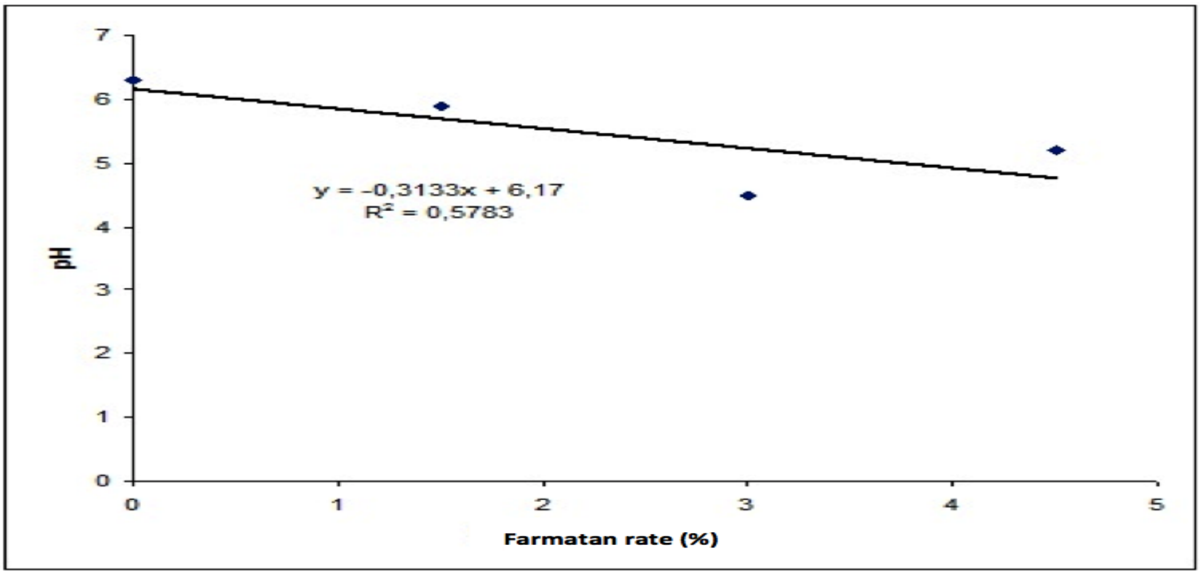

Figure 3. The relationship between Farmatan rate and silage pH.

The effects of Farmatan on gas production kinetics, metabolizable energy, and OM digestibility (OMD) of alfalfa silage were statistically significant (P<0.001; Table 3).

Table 3. Silage additive Farmatan's impact on *in vitro* gas production kinetics, metabolizable energy, and organic matter digestibility level of alfalfa silage.

Parameters	Control	% 1.5	% 3.0	% 4.5	SE	P
c	0.059	0.059	0.062	0.064	0.002	NS
a (ml)	3.61[a]	3.61[a]	2.54[b]	2.02[b]	0.242	***
b (ml)	67.18[a]	62.53[b]	60.93[bc]	58.73[c]	0.788	***
a+b	70.79[a]	66.14[b]	63.47[b]	60.75[c]	0.837	***
ME (MJ/kg DM)	10.39[a]	9.70[b]	9.80[b]	9.59[b]	0.097	***
OMDL (%)	70.42[a]	65.83[b]	66.53[b]	65.14[b]	0.636	***

[a,b,c] :differences between means indicated by different letters in the same row are important. ***: P<0.001, SE: Standard error, NS: Non-significant, c: b's fermentation rate, a: Amount of gas obtained from easily fermented part, b: Amount of gas obtained from slowly fermented part, ME: Metabolic energy, OMDL: Organic matter digestibility level.

In the analysis of gas production and rumen degradability (Figure 4), it can be seen that the highest potential gas production (b) of alfalfa silage ranged from 58.73 to 67.18 mL. The potential gas production of alfalfa silage from control group was higher than that of the other treatments. Gas production rate (c) was affected by Farmatan addition (P<0.001), ranging from 0.059% to 0.064%. Farmatan reduced the amount of gas produced over time.

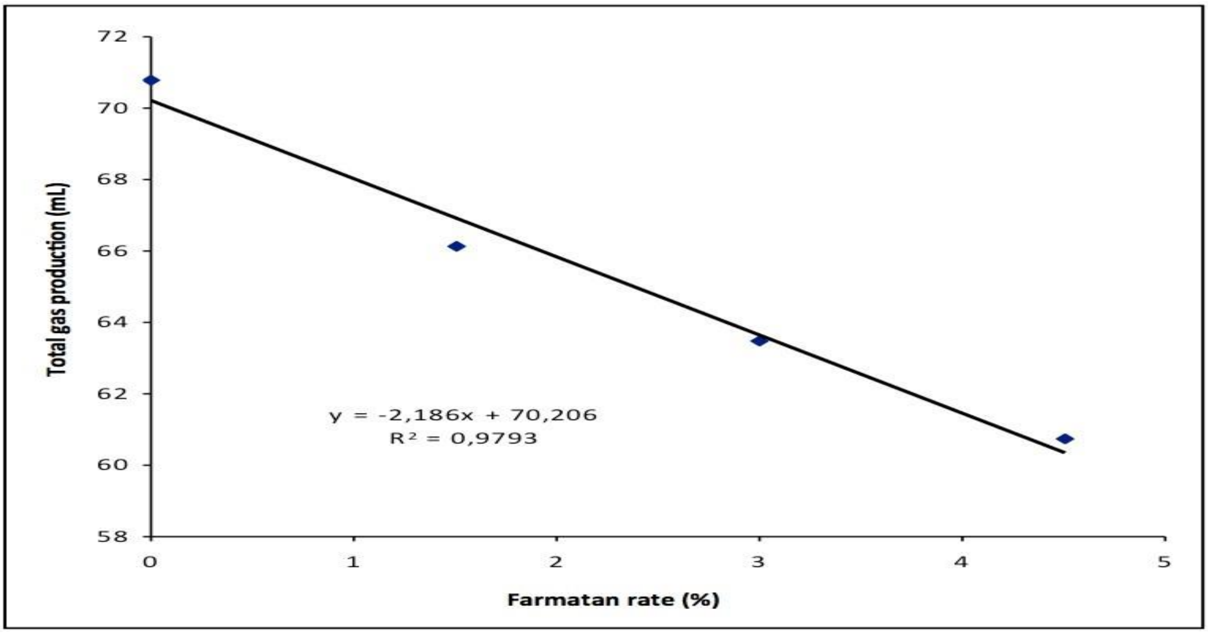

Figure 4. The relation between Farmatan rate and total gas production.

Metabolizable energy (ME) decreased with increasing doses of the additive. One unit of increase in the Farmatan rate led to a decrease of 0.153 units in the total amount of ME.

The OMD of control alfalfa silage was significantly higher than those of the other groups (Figure 5). One unit of increase in Farmatan rate caused 1.00-unit decreases in organic matter digestibility. The reason for the decrease in ME and OMD estimated by using the CP content and 24-h gas production is suggested to be due to the decrease in gas production with the increasing Farmatan levels.

The lowest DM content was observed in control group, whereas the highest was found in the silage supplemented with 4.5% Farmatan. Salawu et al. (1999) reported that the DM content decreased with increasing amounts of tannin added to the silages. Canbolat et al. (2013) observed differences in alfalfa silage, reporting the following mean values: CP - 15.77%, DM - 28.27%, crude fat - 5.59%, ash - 5.92%, NDF - 48.49%, ADF - 30.62%, and pH - 5.37.

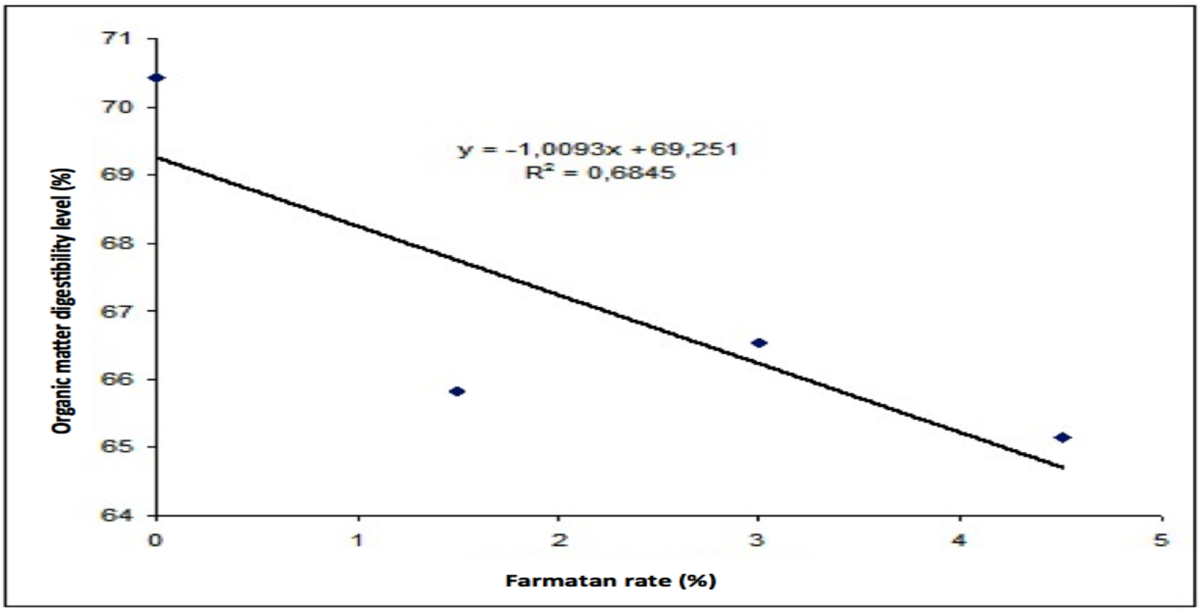

Figure 5. The relationship between the level of Farmatan and organic matter digestibility.

A one-unit increase in Farmatan additive led to an increase of approximately 1.78 units in the DM content of the silage (Figure 1). The reason for this increase in silage DM is the high DM content of the additive. The DM content of control-group alfalfa silage was lower than the 20.44% reported by Ozturk et al. (2006). It was reported that ensilage of alfalfa material with formic acid and enzymes did not cause significant differences in silage composition (Esmail and Muwalla, 1997). Similarly, it was explained that ensilage of alfalfa with Silobac inoculant (*Lactobacillus plantarum* and *Pediococcus pentosaceus*) did not have an impact on the digestibility level of the silage and digestible dry matter intake (Manginelli et al., 2005).

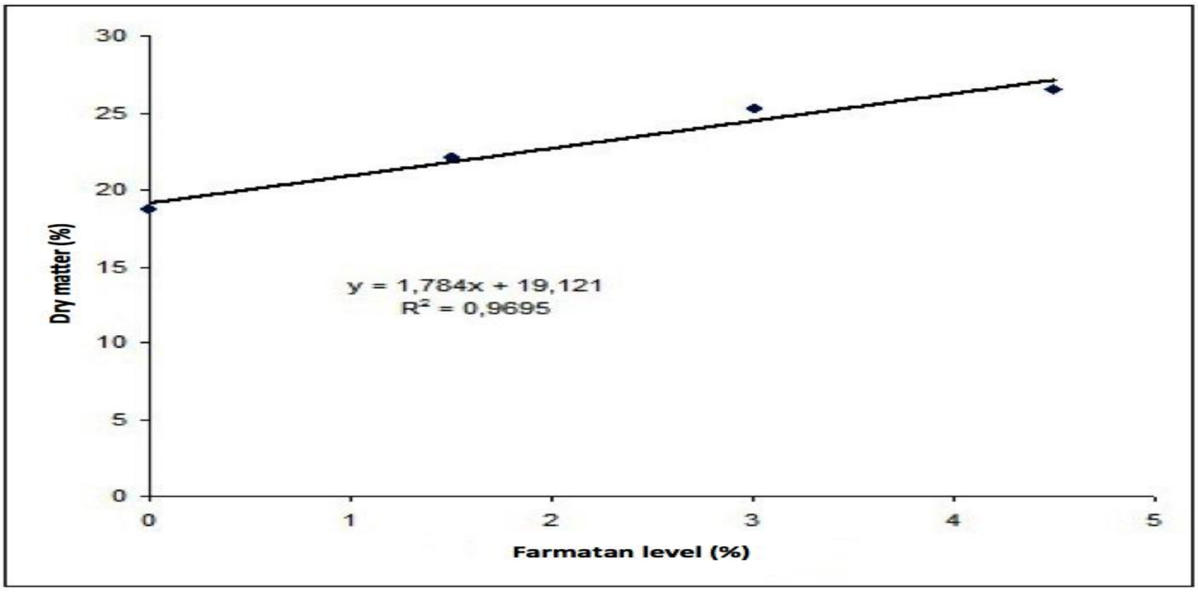

Figure 1. The effect of Farmatan on dry matter content of clover silage.

Kurtoglu and Coskun (2004) reported that ensilage of alfalfa with Pioneer 1174, (*Lactobacillus plantarum* and *Streptococcus faecum*), 5% molasses, 5% barley, and 1.5% salt did not significantly affect the CP, ADF, or ash contents of the silage; however, it had important effects on DM, NDF, pH, and dry matter losses. In another study, it was reported that ensilage of alfalfa material with formic acid, previously fermented filtrate of the material + glucose and fermented filtrate + formic acid decreased the ADF content; however, it increased the CP content (Ruixia et al., 2005).

Kozelov et al. (2008) stated that formic acid application to alfalfa silage increased NDF and *in* *vitro* potential gas production. Ash content values in the present study ranged between 6.97% and 8.80%, which is lower than the 11.64% reported by Ozturk et al. (2006).

The NDF, ADF, and ammonia contents of the silages significantly decreased with the increasing rates of Farmatan (Table 1). We suggest that the reason for the decrease in NDF and ADF is the fact that Farmatan does not include NDF and ADF; the decrease was caused by the dilution effect. Nadeau et al. (2000) reported that alfalfa silages ensiled with cellulase enzyme had its NDF contents reduced. It was reported that silages produced from alfalfa material with molasses and formic acid were better than control group in terms of sensory properties, water content, and pH; molasses and formic acid increased the NDF content (Xian et al., 2004). In the present study, it is suggested that the decrease in ammonia content of the silage was due to the negative impact of Farmatan on proteolytic microorganisms. Generally, the ammonia content in the silage is regarded as an indicator of the proteolysis event occurring during ensilage. Lower ammonia levels as a result of ensilage indicate lower occurrence of proteolysis. Virtanen (1993) reported that proteolysis completely stopped when the silage pH was below 4. The ammonia content of silage with 4.5% Farmatan was approximately 70.11% lower than that of control group. Esmail and Muwalla (1997) indicated that formic acid treatment reduced the ammonia content of alfalfa silage, whereas Ostrowski (1999) reported decreasing ammonia contents with the simultaneous use of bacterial inoculant and sugar. Similarly, it was suggested that ensilage of alfalfa material with tannin reduces the ammonia content (Salawu et al., 1999).

Addition of Farmatan reduced the pH of the silage. The decrease in silage pH is probably due to the acidic structure of Farmatan. The pH of silage material with 3.0% Farmatan was approximately 23.8% lower than that of control group. Low pH has two primary functions in crop preservation: first and foremost, it stops the growth of detrimental anaerobic bacteria; secondly, it reduces the activity of protein-degrading (proteolytic) plant enzymes.

Addition of formic acid and sulfide to alfalfa silage was reported to decrease the silage pH (Marshall et al., 1993). Ohshima et al. (1997) described that ensilage of alfalfa material with fermented filtrate increased the lactic acid content of the silage and reduced its pH. Similarly, it was reported that ensilage of alfalfa material with formic acid decreased the pH of silages (Esmail and Muwalla, 1997). Zhu et al. (1999) explained that ensilage of alfalfa material with cell wall-degrading enzymes reduced the silage pH. Santos et al. (2000), on the other hand, reported that ensilage of alfalfa material with tannic acid did not have a significant impact on the silage pH. It was suggested that as a result of ensiling alfalfa material with acetic acid at various doses, the silage pH decreased, and despite the decrease in proteolytic activity, acetic acid was not an efficient preservative (Djordjevic et al., 2004).

The addition of Farmatan reduced the amount of gas production. The greatest decrease in gas production was observed in the silage with 4.5% addition of Farmatan. Control group had a higher 24-h gas production than the 43.0 mL reported by Tabacco et al. (2006), but was similar to the 56.33 mL reported by Ozturk et al. (2006). It is suggested that the difference in gas measurement values from previous studies depends on the rumen fluid used in incubation and the structural difference of alfalfa silage. Significant decreases ($P<0.001$) in gases obtained from rapidly (a) and slowly (b) degradable parts were observed with increasing rates of Farmatan except for gas production rate (c). A one-unit increase in Farmatan rate caused a 0.389-mL decrease in gas obtained from the easily degradable fraction part and a 1.80-mL decrease in gas obtained from the slowly degradable part. On the other hand, a 2.19-mL decrease was observed in total amount of gas produced. Gas production from the easily degradable fraction ranged between 2.02 and 3.61 mL; from the slowly degradable fraction, between 58.73 mL and 67.18 mL; and total gas production ranged between 60.75 mL and 70.79 mL. The highest production was obtained with control group. The negative impact of Farmatan on gas production is assumed to be due to its antimicrobial effect.

In this study, the ME value of control-group alfalfa silage was higher than the 9.57 MJ/kg DM reported by Tabacco et al. (2006), whereas the OMD value was very close to the 73% obtained by those authors.

Conclusions

Supplementation of alfalfa silage with Farmatan increases the silage quality by increasing its dry matter content and decreasing the pH and ammonia levels. However, it causes significant decreases in metabolizable energy and organic matter digestibility values as determined by the *in vitro* gas production technique. In other words, along with important benefits of Farmatan use, some negative aspects of it are also observed. The point of discussion on this matter is whether significant decreases in protein content along with the benefits provided by Farmatan addition such as decreases in pH and ammonia content compensate for the losses of metabolizable energy and organic matter digestibility. *In vivo* digestion trials may shed light on this issue. If animals like Farmatan-added silage and consume more of it under *in vivo* conditions, then performance losses due to decreases in metabolizable energy and organic matter digestibility may be easily offset.

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