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An Investigation on the Effect of Adding Different Levels of Molasses on the Silage Quality of Pistachio (*Pistachio vera*) by Product and Wheat Straw Mixture Silages

Research Article

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ABSTRACT

The objective of this study was to investigate the effects of adding different levels of molasses (1-5%) on the silage quality, *in vitro* methane production and *in vitro* organic matter digestibility (IVOMD) of pistachio (*Pistachio vera*) by-product and wheat straw mixture silages. For this purpose, silages were prepared with pistachio by-product (85%) and wheat straw (15%) without molasses (control group), and with addition of molasses from 1% to 5% (treatment groups). All the treatments consisted of five replicate silos, and they were prepared in 1.5 L glass jar silos. While silage pH and acetic acid values decreased with addition of molasses levels (P<0.05), silage lactic acid values increased (P<0.05). Butyric and propionic acid were not detected in any of the silages. Addition of all levels of molasses increased *in vitro* methane production, IVOMD and metabolizable energy (ME) values (P<0.05). As a result, fresh pistachio by-product can be ensiled with addition of wheat straw and molasses to produce good quality silage. It can be concluded that this by product can be ensiled well and used as a roughage source with mixture of other roughage sources for animal nutrition in the regions where fresh pistachio by product is available.

KEY WORDS *in vitro* digestion, pistachio by-product, silage.

INTRODUCTION

According to 2015 data, approximately, 25-35 thousand tons of fresh pistachio by-product was obtained from the processing of 144000 tons of pistachio produced in Turkey, one of the top 5 countries in production of pistachio (TUIK, 2013). Utilization of pistachio by-product as a roughage source will prevent pollution as well as converting a waste product to a new income source (Vahmani *et al.* 2006). Nutrient content of this by-products obtained from the processing of pistachio depends on varieties, harvesting time, processing method, leave and stem content. It contains 9-12% crude protein, 21-26% acid detergent fibre (ADF) and 32-37% neutral detergent fibre (NDF) (Bagheripour *et al.* 2008; Valizadeh *et al.* 2009; Mokhtarpour *et al.* 2012). The cheapest and most effective way to store the low dry matter by-product is to ensile it (Mokhtarpour *et al.* 2012). Shakeri *et al.* (2014) reported that the silage of pistachio by-products without additive gives high quality silage, and addition of 12% pistachio by-product silage to the total ration of male calves had no adverse effect. The tannin content in the pistachio by-products may vary due to variety, harvesting time, processing method, and geographic conditions (Bohluli *et al.* 2007). In some studies, tannins reduced the cellulolytic bacterial level and ruminal methane production (Bhatta *et al.* 2009; Goel and Makkar, 2012). This study was performed to investigate the possible utilization of fresh pistachio by-product, which has low dry matter content, no economic value but causes environmental pollution, as an alternative roughage source in the form of silage together with wheat straw supplemented with different levels of molasses.

MATERIALS AND METHODS

Silage preparation and treatments

Wet pistachio (*Pistachio vera*) by-product (PB) was obtained from the private pistachio processing factory in Sanliurfa and wheat straw (WS) and molasses was provided from a local farm. Silages were prepared with PB and WS as a control (85% PB+15% WS, w/w) and with addition of molasses from 1% to 5% (treatment groups, w/w). All the treatments consisted of five replicate 1.5 L glass jars. The jars were stored for 2 months at room temperature and were opened after two months of ensiling.

Analytical methods

The pH values and dry matter contents of the silages were measured after opened the jars. Fresh silage macerated and filtered through two layers of cheesecloth and the pH values of the filtrate were measured with a laboratory pH meter (HI 8314) (Polan *et al.* 1998). After the pH determination, 10 mL of filtrate was acidified and stored at -20 ˚C for silage ammonia nitrogen (NH_3-N) analysis. The NH_3-N content of silages was analyzed according to Kaiser and Piltz (2003) by the Kjeldahl method, and volatile fatty acids (acetic, propionic and butyric acids) and lactic acid of silages were determined by high-performance liquid chromatography (HPLC) according to the method reported by Suzuki and Lund (1980). Determination of silage materials and silage samples dry matter content was done at 105 ˚C for 2 days in the forced-air drying oven. Silage materials and silage samples for chemical analysis were dried at 50 ˚C for 2 days in the forced-air drying oven. The dried silages and silage materials were ground through 1 mm screen in a laboratory mill (Wiley mill) and analyzed for crude protein (CP) and ash content by the AOAC (2005) method. NDF and ADF content were analyzed by the method of Van Soest *et al.* (1991). The condensed tannin (CT) of the pistachio by-product silages and silage materials were determined by the method of Makkar *et al.* (1995). The gas production values of the silages and silage materials were determined through the method described by Menke and Steingass (1988) using four glass syringes as replicate. Rumen fluid for the *in vitro* study was obtained from slaughtered animals thus not requiring any approval about animal use. The IVOMD (% OM) and ME (MJ/kg DM) of silages were calculated using the equations reported by Menke *et al.* (1979). After recording 24 h gas production values, gas inside the syringe was taken by three-way syringe system and total gas was injected into computer-assisted infrared methane gas meter (Sensor Europe GmbH, Erkrath, Germany) and then ruminal methane (CH_4) content was determined as a percentage of the 24 h total amount of gas formed (Goel *et al.* 2008).

Statistical analysis

The statistical analysis of results included one-way analysis of variance using SPSS program (SPSS, 1999). Duncan multiple comparison test was used for comparison of group averages.

RESULTS AND DISCUSSION

Nutrient composition, condensed tannin content, methane production as percentage of total gas production, IVOMD and ME content of wet pistachio by-product, wheat straw and molasses are presented in Table 1. The effects of adding different levels of molasses on nutrient composition of pistachio by-product silages are shown in Table 2. The addition of molasses increased silages DM and CT ($P<0.05$). The addition of 2% or more molasses levels decreased silage CP values ($P<0.05$) and 1% or more molasses levels decreased silage NDF values, while ADF values reduced at 3% or above molasses levels ($P<0.05$). The pH, ammonia nitrogen, organic acids (acetic, propionic, lactic and butyric acid), *in vitro* methane production, IVOMD and ME of silages are presented in Table 3.

The addition of molasses at all levels decreased silage pH and acetic acid value and increased lactic acid content and *in vitro* methane production ($P<0.05$). Finally the addition of molasses at the 4% and 5% levels decreased silage NH_3-N values ($P<0.05$). Propionic acid and butyric acid were not detected in any of the silage treatments. Addition of 2% or more molasses levels increased silage IVOMD and ME values ($P<0.05$).

Crude protein, NDF and ADF values of pistachio by-product determined by Forough and Fazaeli (2005), were 9.2-12.0, 30-36 and 21-28%, respectively, that are consistent with the values obtained in this study. Condensed tannin content of pistachio by-product in current study was similar to the values reported by Boga *et al.* (2013) (20.7-26.3 g/kg DM), and higher than the 18.1 g/kg DM value reported by Ghaffari *et al.* (2014). These parameters can vary depending on variety of pistachio, geographic conditions, soluble sugar content of pistachio, and harvest time (Bohluli *et al.* 2007).

In this study, the increase in silage dry matter with the addition of molasses can be attributed to the decrease in silage pH, due to the inhibition of butyric acid bacteria and of various other microorganisms (Henderson *et al.* 1982), and to higher DM content of molasses.

Table 1 Chemical composition, condensed tannin content, methane production, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) of pistachio by-product, molasses and wheat straw

Item	Wheat straw	Pistachio by-product	Molasses
DM	92.60	19.20	73.62
Ash	11.24	7.86	9.74
CP	4.53	8.76	8.43
ADF	52.76	31.96	-
NDF	79.03	42.19	-
CT	5.93	21.26	8.07
CH_4	13.05	7.11	14.94
IVOMD	41.56	44.41	77.06
ME	5.26	5.99	10.85

DM: dry matter (%); CP: crude protein (% DM); NDF: neutral detergent fibre (% DM); ADF: acid detergent fibre (% DM); CT: condensed tannin (g/kg DM); CH_4: *in vitro* ruminal methane production as a percentage of total gas production (%); IVOMD: *in vitro* organic matter digestibility (% OM) and ME: metabolisable energy (MJ/kg DM).

Table 2 Effect of different levels of molasses on pistachio by-product silage nutrient composition

Item	Molasses level						SEM
	0% (control)	1%	2%	3%	4%	5%	
DM	31.08^{d}	32.08^{c}	32.36^{c}	32.72^{bc}	33.38^{ab}	33.67^{a}	0.20
Ash	9.61^{a}	9.21^{b}	9.42^{ab}	9.45^{ab}	9.50^{ab}	9.79^{a}	0.06
CP	7.95^{a}	7.91^{a}	7.82^{b}	7.75^{bc}	7.68^{cd}	7.63^{d}	0.03
ADF	44.41^{a}	44.11^{a}	43.93^{a}	42.28^{b}	40.35^{c}	40.18^{c}	0.40
NDF	57.97^{a}	56.47^{b}	55.62^{b}	53.79^{c}	53.72^{c}	53.44^{c}	0.38
CT	20.41^{b}	21.54^{ab}	21.74^{ab}	22.00^{ab}	22.40^{a}	23.41^{a}	0.28

DM: dry matter (%); CP: crude protein (% DM); ADF: acid detergent fibre (% DM); NDF: neutral detergent fibre (% DM) and CT: condensed tannin (g/kg DM).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effect of different levels of molasses on pH, organic acids, ammonia nitrogen, IVOMD and *in vitro* methane production content of pistachio by-product silages

Item	Molasses level						SEM
	0% (control)	1%	2%	3%	4%	5%	
pH	4.26^{a}	4.10^{b}	3.98^{c}	3.97^{c}	3.96^{c}	3.94^{c}	0.02
NH_3-N	7.62^{a}	7.22^{ab}	7.63^{a}	7.12^{ab}	6.89^{b}	6.30^{c}	0.11
LA	23.25^{d}	28.47^{c}	34.91^{ab}	37.14^{a}	34.49^{ab}	33.84^{b}	1.05
AA	22.66^{a}	17.08^{b}	14.54^{c}	11.65^{d}	11.50^{d}	12.77^{cd}	0.87
PA	ND	ND	ND	ND	ND	ND	ND
BA	ND	ND	ND	ND	ND	ND	ND
CH_4	7.42^{c}	8.66^{b}	8.65^{b}	9.68^{a}	9.30^{ab}	9.34^{ab}	0.19
IVOMD	44.18^{d}	45.13^{cd}	46.71^{bc}	48.50^{ab}	49.96^{a}	50.30^{a}	0.55
ME	5.82^{d}	6.00^{cd}	6.22^{bc}	6.49^{ab}	6.70^{a}	6.72^{a}	0.08

pH: pH value; NH_3-N: ammonia nitrogen (% NH_3-N/TN); LA: lactic acid (g/kg dry matter (DM)); AA: acetic acid (g/kg DM); PA: propionic acid (g/kg DM); BA: butyric acid (g/kg DM); CH_4: *in vitro* ruminal methane production as a percentage of total gas production (%); IVOMD: *in vitro* organic matter digestibility (% OM) and ME: metabolisable energy (MJ/kg DM).
ND: not detected.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Similarly, it has been reported that homofermentative lactic acid bacteria in a silo converts sugars such as fructose and glucose to lactic acid, and the increased lactic acid content in the environment reduces the loss of dry matter (Kung, 2008). The IVOMD value of dried pistachio by-product was found to be between 69.0 and 74.5 g/kg by Boga *et al.* (2013) and 52.6 g/kg by Noghabi and Rouzbehan (2011). When stored in silos, reduction in the CT level of pistachio by-product was attributed to the oxidation of tannins due to extension of the storage period (Ben Salem *et al.* 2005). However, in this study, CT content increased up to 23.4 g/kg DM in the silage with the increasing molasses level whereas it was 20.4 g/kg DM in silage without molasses.

This difference may result from the addition of different level of molasses on top of silage material in addition to Maillard reaction occurs during ensiling and sample prepetition for tannin analysis.

The significant decreases (P<0.05) in NDF and ADF values with the increasing molasses levels in the silage prepared with pistachio by-product and wheat straw of our study is consistent with the results of Bagheripour *et al.* (2008), who reported that the NDF and ADF values decreased with the extension of storage period of pistachio by-product silage. The reason for this decrease was interpreted as the results of a lower hydrolysis of cellulose and hemicellulose in silo (Yahaya *et al.* 2002). However, in the present study, the decrease may also be the result of gradually increasing molasses level raised DM content and high DM content diluted ADF and NDF content of silages. In a study by Mokhtarpour *et al.* (2012), the decrease in ruminal NH_3-N and NDF digestion was attributed to the diminished disintegration rate of protein in the rumen by creating complex structures of the feed particles with the high levels of condensed tannins. McAllister *et al.* (1994) reported that CT cause a reduction in the nitrogen and NDF digestion in the rumen through binding microorganisms to the rumen or to the nutrients, which reduces bacterial activity that affects plant particles.

In this study, the pH values of all silages prepared with fresh pistachio by-product and wheat straw mixture with increasing molasses were within the range of good quality silages (3.8-4.2) (Leterme *et al.* 1992). The pH values obtained in the presented study were consistent with the results of Valizadeh *et al.* (2009) who determined pH values between 3.90 and 4.22 in fresh pistachio by-product silages prepared by adding molasses at the levels of 1.5%, 3.0% and 4.5% on a dry matter basis. However, Vahmani *et al.* (2007) found pH values between 4.52 and 4.63 in fresh pistachio by-product silage prepared by adding 1.5%, 3.0% and 4.5% molasses, that were lower than the pH value of the control silage (5.27). In the study of Vahmani *et al.* (2007), the ammonia nitrogen value in the pistachio by-product silage prepared by adding urea and molasses decreased, depending on molasses level. Our value, compared with control silage, decreased too. Degradation of protein in the ensiled material depends on the plant protease enzyme as well as the other enzymes that are produced by microorganisms in the environment of the silo. High concentrations of NH_3-N values in the silage (higher than 12-15% NH_3-N/TN) are considered as an indicator of disruption of the proteins, due to the high level of bacterial growth in silo, such as enterobacteria or clostridium (Kung, 2008).

Tannins in silos, in an environment close to neutral pH leads to a reduction of the ammonia nitrogen concentration in the silage through the formation of complexes with soluble proteins, thus preventing the degradation of proteins by microorganisms. In this way not only the loss of nitrogen in silage is prevented but also the silage quality is increased (Santos *et al.* 2000). Acetic acid of silages decreased with the increasing level of molasses, compared with the control group.

In silo the acetic acid production occurs as the result of cleavage of proteins, organic acids and carbohydrates by proteolytic bacteria (Kung, 2008). In this study, the reduction (P<0.05) of acetic acid ratio in the silages prepared with molasses may result from either the formation of complexes of the tannins with proteins, or from the antimicrobial activity of the tannins, which is present in pistachio by-product.

In this study, the *in vitro* CH_4 production in the fresh pistachio by-product silages, prepared by adding 15% wheat straw increased with the increasing of molasses levels. In some previous studies it has been reported that tannins reduce the level of cellulolytic bacteria and ruminal methane production (Bhatta *et al.* 2009; Goel and Makkar, 2012). It was claimed that tannins suppress the rumen protozoa and thus reduce the formation of methane (Moss *et al.* 2000). However, in this study, the increase in the methane gas production with the increasing molasses may be due to the higher methane gas production potential of molasses which contains readily fermentable carbohydrates. In this study, IVOMD and ME values increased significantly (P<0.05) along with the methane gas formation in the fresh pistachio by-product and wheat straw mixture, depending on the increase in the level of molasses added to the silage. The increased IVOMD values of the silages with the increasing molasses may depend on the higher digestibility of the molasses. In addition, the determination of the different nutrients, the tannin content and the IVOMD value of previous studies, in which the silages were prepared with pistachio by-product without wheat straw, may result from the use of wheat straw as silage material as well as variability in harvesting time, processing method and the proportion of branches and leaves of pistachio by-product (Valizadeh *et al.* 2009). Bagheripour *et al.* (2008) determined the IVOMD and CT content of silages prepared with pistachio by-products as 46% and 0.62% respectively, after 60 days of ensiling time. Our IVOMD value of fresh pistachio by-product silages prepared with 15% of wheat straw and by adding molasses at different levels (1-5%) was similar to the values obtained by Bagheripour *et al.* (2008). Shakeri *et al.* (2013) reported that addition of fresh pistachio by-product at the levels of 6%, 12% and 18% showed no adverse effect on feed intake, blood and urine parameters of male calves, without the darkening of the stool and urine color, which may arise from pigments existing in pistachio by-product. In a study performed by Rezaeenia *et al.* (2012) also no adverse effect of silage prepared with pistachio by-products containing 1.5% of molasses, which incorporated to the dairy cattle rations at the level of 15% of the total mixture was found. This by-product was considered as a roughage source.

CONCLUSION

The results of this study have shown that fresh pistachio by-product with low dry matter can be ensiled with 15% wheat straw. Addition of molasses to the silages at the levels ranging from 1% to 5% can improve its quality as an alternative roughage source, and this by-products can contribute to the economy.

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