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Effect of Rosemary (*Rosmarinus officinalis* L.) Powder Supplementation on Silage Fermentation Characteristics, Silage Quality, and *In Vitro* Digestibility in Corn Silage

Besime Doğan Daş[1,a,*]

[1]*Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease, Şanlıurfa, Türkiye*
[*]*Corresponding author*

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A B S T R A C T

The purpose of this study was to investigate how the addition of rosemary to corn silage affected the quality, fermentation, and *in vitro* digestion of organic matter. In the study, unaltered silage group constituted the control group, while silages prepared by adding 0.5, 1 and 2% rosemary constituted the experimental groups. After 60 days of ensiling, the pH of the opened silages was determined to be in the range of 3.67-3.72. As the amount of rosemary in the silages increased, the levels of ammonia nitrogen (NH3-N/TN) decreased. Compared to the control group, increases in in-vitro organic matter digestion (IVOMD) and metabolizable energy (ME) values were observed in the experimental groups due to the addition of rosemary. In comparison to the control group, the methane gas (CH_4) rate of silages was reduced in the other groups. Meanwhile, the group receiving 2% addition of rosemary had the highest values of acetic acid (AA) and lactic acid (LA) among the supplemented silage groups. But, propionic acid (PA) and butyric acid (BA) were not detected in any of the silage groups. Consequently, it was determined that 1% and 2% of rosemary added to corn silage can enhance the quality of the silage.

[a] *bdas@harran.edu.tr* *https://orcid.org/0000-0003-2163-2632*

Introduction

The quality of silage is affected by many factors such as the quality of the silo feed, silo type, dry matter and easily soluble carbohydrate levels of the silo feed, filling into the silo, cover material, ambient temperature, and closure of the silo. Microorganisms and air leaks, which negatively affect fermentation, affect even easily ensiled corn silage. In this regard, it becomes necessary to use silage additives that will help silage fermentation biology and technology (Kılıç, 1986).

Today, medicinal and aromatic plants have a significant role in world trade. China is the leading country in the world utilizing and documenting medicinal plants, followed by some European countries. In many European countries such as Germany, France, and Italy, treatment with medicinal plants is well established and the costs of most herbal treatments are covered by health insurance.

Because of the rapid increase in the human population, there have been severe problems in the utilization of antibiotics, which are feed additives, to increase the potential of animal production (Nir and Şenköylü, 2000). It has been reported that medicinal plants with secondary metabolites can be preferred as an alternative to antibiotics since the usage of antibiotics in animal nutrition was banned in 2006 (Kowalczyk et al. 2013) and therefore it started to be implemented in Turkey as well. The phenolic and flavonoid content of plants is particularly important for the health of the rumen and animal productivity, according to studies on the nutrition of ruminants (Lee et al., 2017; Patra et al., 2006; Rochfort et al., 2008). Dohi et al. (1997) reported that feed intake and thus animal productivity increased in animals fed with plants containing phenolic compounds. Santos-Neto et al. (2009) and Frozza et al. (2013) reported that these compounds have antioxidant and antimicrobial effects, and that flavonoids and phenolic compounds also control nutritional stresses such as rumen fermentation, bloating and acidosis (Paula et al., 2016; Seradj et al, 2014), and that phenolic compounds both promote fermentation in silage and provide an aromatic taste (Gülümser et al., 2022).

Rosemary (*Rosmarinus officinalis* L.) is an essential medicinal and aromatic plant from the *Lamiaceae* family (Ali et al., 2000). Many studies have been conducted on the effects of secondary metabolites of rosemary. It has been reported that rosemary has anticancer (Bai et al., 2010; Valdes et al., 2012; Sanchez-Camargo et al., 2014), insecticidal, antimicrobial (Jordan et al., 2013) and antioxidant (Hussain et al., 2010) effects. Rosemary (*Rosmarinus officinalis* L.) is cultivated as an ornamental plant in regions of our country with Mediterranean climate characteristics (Çelik and Gül, 2016).

This study was out to ascertain how rosemary (*Rosmarinus officinalis* L.) affected the quality, features of fermentation, and *in vitro* digestibility of silages prepared by supplementing rosemary (*Rosmarinus officinalis* L.) to corn silage at different rates (0, 0.5, 1, and 2%) as an antimicrobial additive.

Material and Method

In this study, corn plant was used as silage raw material. The study's control group consisted of corn plants without additions, whereas trial groups were made up of additives containing 0.5%, 1%, and 2% of rosemary powder (R). Each silage group was prepared in 4 replicates and compressed and siled in 1.5 liter glass jars. The silages that had been stored in a dark room were opened after sixty days. After evenly removing 25 g of silage samples from each jar, 100 ml of purified water was added, and the mixture was stirred for two minutes. The pH values of the silage liquid obtained by filtering the liquid in the blender were measured and recorded with a pH meter (WTW 7310). In addition, silage liquid was put into 10 ml centrifuge tubes for analysis of certain volatile fatty acids and ammonia nitrogen. The tubes designated for the analyses of volatile fatty acids (VFA) and lactic acid (LA), and ammonia nitrogen analysis, respectively, received 0.25 ml of 25% metaphosphoric acid and 0.1 ml of 1M HCl before being placed in the deep freezer (-18ºC) until the analysis. Silage samples have been analysed for ammonia nitrogen using the technique described by Broderick and Kang (1980). Using the technique described by Suzuki and Lund (1980), the LA and VFA analysis was carried out. For this purpose, high performance liquid chromatography device (Shimadzu L.C-20 AD HPLC pump, Shimadzu SIL-20 ADHT Autosampler, Shimadzu SPD M20A Detector (DAD), Shimadzu cto-20ac Colum oven, Icsep Coregel (87H3 colon) were used. Aerobic stability testing (CO_2 formation values) of opened silage samples was performed according to the procedure described by Ashbell et al. (1991).

The silages obtained for the study were subjected to analyses of dry matter (DM), crude ash (CA), and crude protein (CP) in accordance with AOAC (2005). Analyses of acid detergent soluble fiber (ADF) and neutral detergent soluble fiber (NDF) were conducted using the methodology described by Van Soest (1991). Fresh silage materials and silages that had undergone 60 days of fermentation were both dried at room temperature, ground and made ready for all analysis. The approach described by Menke et al. (1988) was used to apply the in vitro organic matter digestibility (IVOMD), metabolizable energy (ME), and methane (CH_4) contents of silages techniques. Using the obtained 24-hour gas production amounts, IVOMD and ME values of the silages were calculated with the equation reported by Menke et al. (1979). Using the technique described by Filya et al. (2000), yeast and mold analyses of silages were conducted.

The data were subjected to using the General Linear Models procedure of the SPSS 25.0 statistical program. The data were analysed using One Way Analysis of Variance (One-Way ANOVA), and group averages were compared using the Duncan multiple comparison test..

Article 8(k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" states that HADYEK approval is not needed for this research.

Results and Discussion

The corn plant and Rosemary (*Rosmarinus officinalis* L.) powder, which were both included in the study, had their nutritional analysis results summarized in Table 1.

The nutrient contents, pH, NH_3-N/TN and CO_2 values of the silages prepared by supplementing rosemary at different rates (0%, 0.5%, 1% and 2%) to corn plants are presented in Table 2.

Upon examination of Table 2, it became evident that CP, CO_2, and NH_3-N/TN levels of the silages varied statistically significantly (P<0.05) among the groups. In terms of DM, CA, ADF, NDF, and pH values, there were no statistically significant differences between the groups (P>0.05).

Table 1. Crude nutrient contents of corn plant and rosemary utilized in the study.

	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Corn	27.44	7.33	7.63	30.24	59.05	47.1.84	6.99	13.01
Rosemary	92.24	7.25	8.31	31.05	40.19	31.49	4.59	11.88

DM: Dry matter, %; CA: Crude ash, DM%; CP: Crude protein, DM%; ADF: Acid detergent insoluble fiber, DM%; NDF: Neutral detergent insoluble fiber, DM%; IVOMD: *In Vitro* organic matter digestion, %; ME: Metabolizable energy, MJ/kg DM; CH_4: *In Vitro* methane gas, %.

Table 2. Nutrient contents, pH, NH_3-N/TN and CO_2 values of silages prepared by supplementing rosemary to corn plants.

Groups	DM	CA	CP	ADF	NDF	pH	NH_3-N	CO_2
Control	25.904	6.920	7.714^{b}	30.627	60.158	3.690	9.705^{a}	3.718^{a}
% 0.5 R	25.851	7.217	7.848^{b}	31.000	60.480	3.715	8.068^{b}	3.002^{ab}
% 1 R	25.430	7.202	8.569^{a}	31.462	59.447	3.700	7.237^{b}	2.753^{b}
% 2 R	26.088	7.362	9.011^{a}	31.319	59.808	3.668	7.133^{b}	1.197^{c}
SEM	0.232	0.132	0.170	0.220	0.258	3.690	0.303	0.268
P	0.819	0.729	0.004	0.594	0.571	0.084	< 0.01	0.000

$^{a-c:}$ Values with different letters in the same column were found to be different (P<0.05); DM: Dry matter, %; CA: Crude ash, DM%; CP: Crude protein, DM%; ADF: Acid detergent insoluble fiber, DM%; NDF: Neutral detergent insoluble fiber, DM NH_3-N/TN: Ammonia nitrogen; CO_2: Carbon dioxide, g/kg DM; R: Rosemary, SEM: Standard Error of Mean.

Table 3. The effect on fermentation characteristics of silages prepared by supplementing rosemary to corn plant.

Groups	TYM	LAB	IVOMD	ME	CH_4	LA	AA	PA	BA
Control	7.408	7.585	40.830^{b}	6.929^{b}	14.395^{a}	7.813^{d}	2.256^{d}	-	-
% 0.5 R	7.290	6.838	46.723^{a}	6.858^{b}	14.220^{a}	8.903^{c}	2.833^{c}	-	-
% 1 R	6.798	6.625	47.493^{a}	7.255^{ab}	13.010^{ab}	11.016^{b}	2.977^{b}	-	-
% 2 R	6.043	6.603	47.179^{a}	7.529^{a}	11.198^{b}	12.433^{a}	3.813^{a}	-	-
SEM	0.290	0.227	0.730	0.9010	0.456	0.456	0.168	-	-
P	0.356	0.409	0.000	0.011	0.027	0.0000	0.0000	-	-

$^{a-d}$: Values with different letters in the same column were found to be different (P<0.05); Total Yeast-Mold (TYM): log10/cfu/gr; LAB: Lactic Acid Bacteria, cfu/g; IVOMD: *In Vitro* organic matter digestion, %; ME: Metabolizable energy, MJ/kg DM; CH_4: *In Vitro* methane gas, % LA: Lactic acid, g/kg DM, AA: Acetic acid asit, g/kg DM, PA: Propionic acid, g/kg DM; BA: Butyric acid, g/kg DM, SEM: Standart Error of Mean.

Table 4. Correlation of chemical analysis in silages.

		pH	NH_3-N	LA	AA	Yeast-Mold	IVOMD	ME	CH_4	CO_2
pH	PC	1	.062	-.279	-.020	-.148	-.455	.052	-.013	.284
	P		.819	.381	.950	.583	.077	.849	.961	.287
NH_3-N	PC		1	-.871**	-.848**	.328	-.147	-.481	.376	.522*
	P			.000	.000	.214	.588	.059	.152	.038
LA	PC			1	.939**	-.374	.427	.932**	-.663*	-.512
	P				.000	.231	.166	.000	.019	.089
AA	PC				1	-.462	.216	.930**	-.603*	-.303
	P					.130	.500	.000	.038	.338
Yeast-Mold	PC					1	.002	-.039	.078	-.068
	P						.994	.886	.775	.802
IVOMD	PC						1	.454	-.249	-.248
	P							.077	.353	.355
ME	PC							1	-.586*	-.283
	P								.017	.288
CH_4	PC								1	.283
	P									.288
CO_2	PC									1

PC: Pearson correlation, *: Correlation is significant at 0.05 level; **: Correlation is significant at 0.01 level; NH_3-N/TN: Ammonia nitrogen; LA: Lactic acid g/kg DM; AA: Acetic acid g/kg DM; IVOMD: *In vitro* organic matter digestion %; ME: Metabolizable energy, MJ/kg DM; CH_4: *In vitro* methane gas (%); CO_2: Carbon dioxide g/kg DM.

There was no difference (P>0.05) in the values of DM, CA, ADF, NDF and pH of the silages prepared by supplementing rosemary to corn plant. This may be due to the low levels of rosemary supplementation. Similarly, Özelçam and Daşıkan, (2017). In their study to determine the effect of carvacrol addition to corn silage on aerobic stability, pH and visual mold growth, they reported that carvacrol addition to corn silage (0, 200, 400, 800, 1600 ppm) did not affect the dry matter and pH values. An increase was seen between the groups when the CP levels of the silages were evaluated, paralleling the rise in rosemary supplementation (P<0.05). This increase was due to the addition of rosemary (8.31% DM) which has high protein content. The control group in the study had the highest NH_3-N/TN value (9.705), while the group that added 2% rosemary had the lowest NH_3-N/TN value (7.133) when the silage NH_3-N/TN values were compared to it (P<0.05). It was observed that the lowest NH_3-N/TN amounts of corn silages were in 1% R and 2% R groups (P<0.05). The supplementation of rosemary to corn silage was effective in preventing proteolysis, thus NH_3-N/TN amounts decreased. In addition, the highest CP content was determined in the 1% R (8.569% DM) and 2% R (9.011% DM) groups, confirming this finding. Similarly, Canbolat et al. (2010) found that adding thyme oil to corn silage significantly reduced the amount of ammonia (NH3). However, low pH of silage is closely related to LA production and increased LA production rate decreases proteolysis activity (Uçar and Garipoğlu, 2015; Muck, 1996). However, silage ammonia nitrogen values were determined to be lower than 10%. The fact that these values are lower than 10% indicates that the fermentation quality of silages is high (Kaiser and Piltz, 2003).

When CO_2 of the silages obtained was analysed, the lowest CO_2 value (1.197 g/kg DM) was detected in the group with 2% rosemary supplementation compared to the control (3.718 g/kg DM). With the increase in rosemary supplementation, a decrease in CO_2 value was observed and aerobic stability of silages increased (P<0.05). Similarly, Önenç et al. (2019) examined how thyme and cumin essential oils affected alfalfa's fermentation quality and aerobic stability; they discovered that 650 mg/kg of these oils improved silage fermentation and improved aerobic stability. Similarly to Özelçam and Daşıkan (2017) have been found that carvacrol addition to corn silage were increased aerobic stability.

The correlation of the chemical analyses of the silages prepared by supplementing rosemary at different rates (0.5%, 1% and 2%) to corn plant is presented in Table 4.

In the study, while total yeast mold and LAB values of silages prepared by adding rosemary to corn plants were determined to be statistically insignificant (P>0.05), differences between groups in IVOMD, ME, CH_4, LA and AA values were observed to be statistically significant (P<0.05). IVOMD and ME values of silages increased with rosemary supplementation, while CH_4 decreased

(($P<0.05$). The highest IVOMD value (47.493%) was realized with 1% rosemary, the highest ME value (7.529%) with 2% rosemary and the lowest *in vitro* CH_4 value (11.198%) with 2% rosemary addition. IVOMD and silage CH_4 values were found to be negatively correlated when Table 4 was examined (R: -.249). Examining the silage CH_4 values, all experimental groups had lower values than the control group ($P<0.05$). The presence of rosemary is thought to reduce the total number of bacteria and cellulolytic bacteria in the rumen fluid, suppress rumen protozoa, and hence lessen the production of methane gas (Ghasemi et al. 2012; Moss et al., 2000).

When the LA and AA values of the silages were analysed, LA and AA values increased with the increase in rosemary compared to the control group ($P<0.05$). The highest values were observed as 12.433 and 3. 813 g/kg DM in the 2% rosemary supplemented groups, respectively. The high values of these values were related to the low pH values (range 3.8-4.2) of the silages (Ergül et al. 2013), which increased the lactic acid bacteria and increased the LA value. The low pH values in the study resulted in high lactic acid levels in all silage groups.

When Table 4 is analysed, the positive correlation (R:.939) between LA and AA confirms this statement. In well ensiled silages, lactic acid bacteria develop rapidly and become dominant in fermentation. These LAB produce high amounts of LA and AA by consuming the carbohydrates in the structure of corn and rapidly decrease the pH level of the environment (Filya 2001).

In the present study, acetic acid contents obtained from silages increased aerobic stability by decreasing CO_2 values and partially reduced yeast and mold growth (Table 2, 3). The reason for this is due to the antimicrobial effect of AA; it has been reported in studies that AA inhibits yeast mold growth and CO_2 formation (Danner et al., 2003; Schmidt et al., 2009).

There were no PA or BA in any of the study's groups. In the case of a pH value of 3.80-4.20, it is not probable for butyric acid producing bacteria to multiply and display activity since lactic acid bacteria are dominant. While these microorganisms are active at neutral and basic pH values, they lose their activity rapidly at low pH values. The absence of butyric acid in the silage groups is a sign that the silages were of a satisfactory quality (McDonald P, 1981). It was also reported that BA was not detected in well-fermented silages (Kung et al., 2018).

Conclusion

As a result of the study, the supplementation of rosemary (0.5%, 1% and 2%) to corn silage at different rates increased IVOMD, ME and LA values in silages, decreased CO_2 formation and increased aerobic stability. Due to these positive effects, it was concluded that rosemary powder can be added to the silage at 1% and 2% levels while making corn silage, and it would be worthwhile to determine the effect of rosemary on the quality and fermentation parameters of silages by increasing the additive dose in future studies and to determine the effect on feed consumption and digestion degree under *in vitro* conditions.

Authors contributions

BDD created the original hypotheses, planned the experiments, gathered the data for the investigation, carried out the statistical analysis, worked with the team to evaluate the findings, and drafted and edited the report.

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