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Effect of Silage from Five Varieties of Corn Forage on Feed Intake, Digestibility, and Ruminal Parameters in Sheep

Research Article

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ABSTRACT

In this study, the effect of silage made from five corn forage cultivars was investigated on feed intake and digestibility, and ruminal parameters in sheep. For this purpose, five Kermani rams were used with an average weight of 40.1 ± 2 kg. The experiment was conducted as a Latin square design (5×5) in five 21-day periods. Initially, 300 kg of each of the 5 corn forage varieties Dracma, Lavida, Sagunto, 704, and Korduna, silage was prepared in 90 × 45 cm plastic bags. Nearly after 60 days, the silages were evaluated. Silages were used in experimental diets with 40% corn silage, 20% dry alfalfa hay, and 40% concentrate (based on 100% dry matter). The dry matter intake (DMI) and other nutrients were not affected by the experimental diets. The digestibility of organic matter, ether extracts (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were affected by the type of silage in the experimental diets ($P<0.05$). The digestibility was higher in diets containing Sagunto and 704 silage than the other silages ($P<0.05$). The NH_3-N concentrations were higher in Sagunto cultivar than the Lavida cultivar and other corn silage cultivars. The number of Holotrich and Entodinium protozoa had the highest value ($P<0.05$) in the ruminal fluid in the diet of Sagunto cultivar. The type of silage had no significant effect on the pH, ruminal volatile fatty acids, blood parameters and microbial protein synthesis. The Lavida silage had the lowest ADF content, but the Lavida silage diet had the lowest organic matter (OM) digestibility. Lavida silage had the lowest ADF value, but the dietary OM digestibility of this silage was the lowest.

KEY WORDS digestibility, Kermani sheep, microbial protein, ruminal fermentation, ruminal parameters.

INTRODUCTION

Feeding has the highest cost in livestock production (Verbeke *et al*. 2015). Feed is a major factor in the breeding of domestic animals and plays an important role in animal health and profitability (Cilek and Gotoh, 2015). Globally, agriculture accounts for 70 percent of all surface and groundwater withdrawals, mainly for intensive feed and forage production (FAO, 2021). Corn forage is considered an important product as animal feed due to its quantity and quality. Corn forage is rich in soluble carbohydrates and starch and the yield of corn forage is about 80 to 100 tons per hectare (Khan *et al*. 2015) and has suitable characteristics for preparing silage (McDonald *et al*. 2011). Silage preparation is a common method of storing forage at high humidity. The basis of the silage preparation process is the conversion of water-soluble carbohydrates to organic acids by lactic acid bacteria. Silage is produced by the fermentation of stalks, leaves and cobs of corn plants or other green fodder by anaerobic fermentation. This feed can be stored for a long time and has a lot of energy (Johnson, 2005). Corn forage has a high dry matter (DM) content and a low

buffering capacity, thus, its lactic acid production is higher compared to silage from other gramina grasses (Ferraretto *et al*. 2018). Silage varieties have many differences in chemical composition (Grant and Ferraretto, 2018; Velho *et al*. 2020). It was hypothesized that Iranian 704 corn fodder does not differ from foreign varieties of corn in terms of chemical composition and animal nutrition quality. Therefore, the aim of this study was to compare the effect of silage of five corn cultivars including Lavida, Dracma, Cordona, Sagunto and Iranian cultivar 704 on silage characteristics, feed intake, digestibility and ruminal parameters and blood metabolites in Kermani sheep.

MATERIALS AND METHODS

Study site

The experiments were carried out at the sheep breeding station of Shahid Bahonar University of Kerman, Kerman, Southeast of Iran (latitude 30 ˚15`.1910``N, longitude 57 ˚6.2040``E). The experiment lasted 105 days (in addition, 6 days for the adaptation of the rams to the metabolic cages) including five 21-day periods from 5th April to 25th June.

Ensiling materials and measurements of silage characteristics

Maize varieties include native 704 and Lavida and Dracma (Switzerland), Cordona (America), and Sagunto (Spain) cultivated in the experimental field at Shahid Bahonar University of Kerman in May (latitude 30˚ 150N, longitude 57˚ 010E, altitude 1755 m), Kerman, Iran and harvested in mid-September. On the day of silage making, the corn plants were chopped by a conventional forage chopper to lengths of 8 mm. The chopped forages were ensiled without any additive in nylon bags with a size of 90 × 45 cm and stored in a room with 20 – 25 ˚C ambient temperature for 60 days of ensiling.

Immediately after sampling, 200 mL of distilled water was added per 20 g of corn silage and thoroughly mixed for one minute. The pH of the sample was measured with a digital pH meter (AZ 8686, Taiwan) (Hattori *et al*. 2008). The ammonia nitrogen was also measured. Briefly, 40 g of the silage sample was mixed with 360 mL of distilled water for three minutes and the resulting solution was filtered through filter paper (Whatman, Maidstone, U.K.) and 100 mL of the extract was transferred to a Kjeldahl apparatus (Buchi K 370, Germany) to determine ammonia nitrogen (Filya, 2003).

Sensory evaluation of silage

Sensory evaluation of silage was performed by four experts (Karasahin, 2014) with four replications per each silage type based on odor (maximum 14 points), texture structure (maximum 4 points) and color (maximum 2 points).

Chemical composition, *in vitro* gas production

Samples of feed ingredients, feed refusal, and fecal samples were dried in a forced-air oven at 65 ˚C for 48 h and were then ground to a size of 1 mm before analysis. Standard methods were used to measure the dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extract (EE) of feed, feed residues and fecal samples (AOAC, 2005). Non fiber carbohydrate (NFC) was determined by the method of DePeters and Arosemena (2000).

Gas test

Gas Production was recorded at 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 hours of incubation with Lutron digital pressure gauge (model Pm-9100). Using the standard curve, the pressure numbers were converted to gas volume and the equation $P=b\ (1-e^{-ct})$ was used to determine the gas production parameters (Ørskov and McDonald, 1979). In this equation, the b is the gas produced from the water-insoluble but fermentable fraction (mL per 200 mg/DM); c is the gas production rate (mL/h); t is the incubation time in hours and p is the total volume of gas produced (mL/h) (Lopez *et al*. 2007). Fitcurve software was used to estimate the kinetic components of gas production (a, b, c).

Experimental design, rams and diets

Five 2-year-old Kermani rams (mean live weight of 40.1±2.1 kg) were assigned to five treatments in the form of a Latin square design. The animals were maintained according to the guidelines of Iranian Council on Animal Care (1995). The experiment had five periods and each period consisted of 21 days including 14 days for adaptation and 7 days for sampling. Animals were housed indoors under continuous light in individual metabolism cages (0.75 m×1.5 m), which were equipped with a separate urine and fecal collection system. Rams were fed at appetite level (10% remaining). Animals had free access to water. All diets were iso-energetic and iso-nitrogenous (Table 1) and consisted of 60% forage and 40% concentrate and were fed as a total mixed ration (TMR) at 08:00 and 18:00 h every day.

Intake and digestibility

The DM intake was recorded daily and feed bunk contents were analyzed for DM, OM, CP, and NDF, using similar procedures to those of fecal samples at 0 and 24 h after feeding on d 15 to 19. Intakes of DM, OM, CP and NDF were determined by subtracting the amount of distributed and refused. The total fecal collection method was used for the calculation of DM and other nutrients digestibility (Rymer, 2000).

The DM digestibility was calculated as follows (Rymer, 2000).

DMD= (DM intake-fecal DM excreted) / (DM intake) × 100 Equation 1

Digestibility was calculated for other nutrients such as OM, CP, EE and NDF by replacing them with DM intake and excreted in equation 1.

Rumen parameters

Rumen fluid sampling was performed using a gastric tube connected to the suction device on the last day of each period, at the zero time (before feeding) and at times of two, four, six, and eight hours after feeding. Samples of rumen fluid were strained through four layers of cheesecloth and pH was measured immediately by a digital pH meter (Model AZ 8686). Five ml of ruminal fluid was mixed with 0.2 mL of 50% sulfuric acid to determine ammonia nitrogen. Rumen ammonia nitrogen concentration was measured using the phenol-hypochlorite method (Weatherburn, 1967).

Ciliated protozoa

To count ciliated protozoa, 10 mL of strained ruminal fluid was maintained with 10 mL of MFS (methylgreen-formalin-saline) solution (Ogimoto and Imai, 1981). Protozoa were counted four times in each sample by a DQ neubauer slide using an Olympus CH-2 microscope with a magnification of 1500.

Gas chromatography

Gas chromatography (Chorompack, Model CP-9002, Chrompack, EA Middeburg, Netherlands) was used to determine the concentration of volatile fatty acids (VFAs) in ruminal fluid. Rumen microbial nitrogen production was calculated based on grams of protein per day based on urinary purine derivatives using Equation 2 (Denek and Can, 2006).

Microbial nitrogen production= (x(mmol/day)×70) / (0.116×0.83×1000) Equation 2

Where:
x: rate of purine uptake (mmol/day).

Blood samples

Blood samples were taken from animals on the fifth day of sampling, approximately three hours after the mand morning meal. Blood was drawn from a Jugular vein with a syringe. Blood samples were then transferred into a test tube (TBI) containing an anticoagulant (EDTA).

Test tubes containing five ml of blood were transferred to the laboratory. The samples were placed in a centrifuge (Pars Azmoun Co., Iran) at 5000 rpm for five minutes to separate the plasma.

Plasma was then transferred into micro tubes using a micropipette. Samples were stored at -20 ˚C until the experiment.

Statistical model

Data on the chemical composition of silages were statistically analyzed using a completely randomized design. The following statistical model was used (Equation 3):

$Y_{ijk}= \mu + T_i + e_{ijk}$ Equation 3

Where:
Y_{ijk}: each of the observations.
μ: total mean.
T_i: effect of corn silage type (Dracma, Lavida, Sagunto, 704 and Korduna silages).
e_{ijk}: residual variance.

Tukey test at 5% level was used to compare the means.
In the animal experiment data, a 5 × 5 latin square design was used and the statistical model was as follows (equation 4):

$Y_{ijkl}= \mu + T_i + \gamma_j + \delta_k + e_{ijkl}$ Equation 4

Where:
Y_{ijkl}: each of the observations.
μ: total mean.
T_j: effect of corn silage type (Dracma, Lavida, Sagunto, 704 and Korduna silages).
γ_j: random effect of animal (Sheeps A, B, C, D and E).
δ_k: effect of the period (Periods 1, 2, 3, 4 and 5).
e_{ijkl}: random error.

SAS (2005) software was used for data analysis. Tukey test at 5% level was used to compare the means (Cilek and Gotoh, 2015).

RESULTS AND DISCUSSION

The DM values of corn silages were significantly different after 60 days of ensiling (Table 2, P<0.01). The Cordona and Lavida silages had the highest DM values, due to delayed forage harvest (Oney Colton *et al*. 2018). Corn silage of Lavida cultivar and 704 had the highest CP concentration and the silage of Sagunto cultivar had the lowest value (P<0.05).

Table 1 Components and chemical composition of experimental diets (DM %)

Components of experimental diets	Experimental diets based on corn silage cultivars				
	704	La Vida	Dracma	Cordona	Sagunto
Corn silage	40.0	40.0	40.0	40.0	40.0
Alfalfa	20.0	20.0	20.0	20.0	20.0
Barley grain	10.0	10.0	10.0	10.0	10.0
Corn grain	14.0	8.0	13.5	14.0	14.0
Soybean meal	11.0	12.5	12.5	12.0	12.5
Wheat bran	2.5	1.0	1.5	1.5	1.0
Dicalcium phosphate	0.6	0.6	0.6	0.6	0.6
Crushed limestone	1.0	1.0	1.0	1.0	1.0
Mineral and vitamin supplement[1]	0.7	0.7	0.7	0.7	0.7
Salt	0.2	0.2	0.2	0.2	0.2
Chemical composition of experimental diets					
DM (%)	50	57.0	50.0	55.0	52.0
OM (%)	91.4	92.5	90.4	90.4	90.4
CP (g/kg)	132.3	132.7	137.7	133.7	130.1
EE (%)	2.4	2.2	1.8	1.85	2.0
NDF (%)	38.7	37.4	38.1	38.0	37.9
ADF (%)	24.1	21.6	24.1	24.6	26.3
Ca (g/kg)	7.9	7.9	7.9	7.9	7.9
P (g/kg)	4.1	4.8	4.1	4.1	4.1
NFC (g/kg)[2]	39.2	39.3	39.4	39.2	40.0
ME (MCal/kg)	2.23	2.27	2.23	2.22	2.19

[1] Each kilogram of supplement contains: vitamin A: 600000 IU; vitamin D_3: 200000 IU; vitamin E: 200 mg; Mn: 2200 mg; Ca: 195 mg; Zn: 300 mg; P: 80 g; Mg: 21000 mg; I: 12 mg; Fe: 3000 mg; Cu: 300 mg; Co: 100 mg and Se: 1.1 mg.
[2] NFC= 100 – (ASH %+CP %+EE %+NDF %) (DePeters and Arosemena, 2000).
DM: dry matter; OM: organic matter; CP: Crud protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber and NFC: non fiber carbohydrate.

Table 2 Chemical composition of silages of five corn cultivars 60 days after preparation

Chemical composition (DM %)	Corn silage cultivars					SEM	P-value
	704	Dracma	Sagunto	Cordona	Lavida		
DM	29.2^{c}	28.5^{d}	26.8^{e}	34.5^{b}	36.9^{a}	0.10	< 0.01
Ash	6.95	9.19	7.76	9.20	5.93	1.84	0.14
CP	7.08^{a}	5.26^{c}	5.34^{d}	6.64^{b}	7.08^{a}	010	< 0.01
EE	2.53	2.15	1.76	2.27	2.81	0.97	0.52
NH_3-N	0.16^{b}	0.16^{b}	0.15^{b}	0.20^{a}	0.21^{a}	0.01	< 0.01
pH	3.60^{d}	3.70^{c}	3.50^{d}	3.82^{b}	3.90^{a}	0.007	< 0.01
NDF	48.7^{a}	47.0^{a}	46.8^{a}	46.6^{a}	38.8^{b}	2.18	0.03
ADF	28.4^{c}	34.0^{b}	38.5^{a}	29.7^{bc}	20.5^{d}	3.54	< 0.01
NFC[1]	34.8	37.0	38.3	35.3	45.5	3.75	0.09
Sensory evaluation[2]							
Smell	10.7	12.0	12.0	14.0	10.7	1.46	0.10
Structure	3.33	4.00	4.00	4.00	4.00	0.52	0.45
Color	1.67	2.00	2.00	2.00	2.00	0.26	0.45
Total score	15.7	18.0	18.0	20.0	16.7	2.08	0.20
***In vitro* gas production**							
Gas production from insoluble fraction (mL)	64.7	50.0	58.5	64.5	55.2	12.4	0.43
Gas production potential (mL)	72.7	59.1	63.0	69.4	57.7	13.9	0.51
Gas production per hour (mL)	0.05	0.05	0.06	0.06	0.05	0.02	0.97

[1] NFC= 100 – (ASH %+CP %+EE %+NDF %) (DePeters and Arosemena, 2000).
[2] (McDonald *et al*. 2011).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The highest concentration of NH_3-N was in Cordona and 704 cultivars and the lowest value was in Dracma, Sagunto and Lavida cultivars. The high concentration of NH3-N in those silages was probably due to their high pH values.

This is in line with the findings of Asadi Alamouti *et al*. (2004) who concluded that high pH in silage, increased the Clostridium activity which may have increased the degradability of CP and increased the concentration of NH_3-N.

The highest pH value was in Lavida cultivar silage and the lowest value was in 704 and Dracma Cultivars (P<0.05). The high pH of Cordona silage was due to the high DM of Cordona silage. Less soluble carbohydrates were available for fermentation and production of volatile fatty acids with increasing silage DM (McDonald *et al*. 2011). The silages had significant differences in NDF and ADF (P=0.03 and P<0.01, respectively), and the lowest values were in Lavida silage cultivar (P<0.05), because this silage had a large amount of cob (grain) that reduced fiber fraction concentrations. Silages did not have significant differences in ash, EE, NSC, sensory evaluation of silages and gas production.

The DM and other nutrients intake were not affected by silages of corn cultivar in experimental diets (Table 3). Forage particle size affects feed intake (Mertens, 1997), but because the corn forages were chopped to the same size and silage particles size was the same, there was no significant difference in DM and nutrient intake. The chemical composition of all five experimental diets was the same, so there were no significant differences in DM and nutrients intake. However, research shows that in addition to forage particle sizes, factors such as the NDF digestibility and the fiber fragility of corn silage affect feed intake in dairy cows (Faichney, 2005).

It was expected OM digestibility to be higher in Lavida silage diets than in the other diets because its NDF concentration was lower than in other silages. But the results showed that the OM digestibility in Sagunto and 704 diets were higher than the other silage diets (P<0.05). The cultivar of Lavida was harvested late and the digestibility decreases by increasing maturity and crystallization of starch in this cultivar (Peyrat *et al*. 2016). The digestibility of EE was higher in diets containing 704 and Sagunto cultivars silage than in other diets (P<0.05), because EE is a part of organic matter, the higher OM digestibility caused the high digestibility of EE.

The NDF and ADF digestibility in Sagunto, Lavida and Cordona silage diets were lower than 704 and Sagunto silage diets, because Lavida and Cordona cultivars were harvested later in 35-36% DM. Delayed harvest increased lignin in stems and decreased fiber digestibility (Darby and Lauer, 2002; Ferraretto *et al*. 2018). The digestibilities of CP and NFC were not affected by corn silage cultivar, due to the same concentration of CP and NFC in the experimental diets.

Rumen pH was not affected by experimental diets at any sampling time (Table 4). The ruminal pH is influenced by some factors such as forage particle size, DM intake and the rate of degradation of dietary carbohydrates (Kristensen *et al*. 2010). However, in this experiment, the DM intake was not affected by the silage types in the experimental diets, because all corn forages were chopped with the same particles size (Sharifi *et al*. 2018). One of the factors influencing daily pH changes is changes in feed intake (Aschenbach *et al*. 2011), and in the current experiment, there was a little change in DM intake. Fermentable carbohydrates were not different in experimental diets (Table 1). Hedayatipour *et al*. (2012) reported that ruminal fluid pH was not significant in sheep fed experimental diets of corn silage replaced with sweet sorghum silage, probably due to the similarity of percentage of concentrates and the same amount of protein and ash.

The ruminal NH_3-N concentration was higher in the Sagunto cultivar silage diet than other silage diets at two hours after feed intake (Table 4, P<0.05). The percentage of degradable proteins in the rumen is one of the factors affecting ruminal NH_3-N concentration (Davies *et al*. 2013). However, in the present study, the percentage of dietary proteins was the same and had no effect on the ruminal NH_3-N concentration (Table 1). Therefore, it was expected the ruminal NH_3-N concentrations were to be the same between treatments (Pinho *et al*. 2017). However, the total number of protozoa in diets containing Sagunto and Cordona silages was higher than other diets (Table 4). The effect of protozoa on the degradability of insoluble proteins in the rumen is greater than that of bacteria (Ushida and Jouany, 1985). Therefore, with more protozoa, more NH_3-N was produced (Chen and Gomes, 1995).

The populations of Holotrich and Entodinium Species and Total protozoa per ml of rumen fluid were affected by experimental diets (Table 4). The population of Holotrichs was lower in the diet of Lavida silage than the other diets, because Lavida cultivar had less water-soluble carbohydrates due to late harvesting (P<0.05). One of the biological characteristics of Halotrichs is the intake of non-starchy carbohydrates and soluble sugars (Williams and Coleman, 1988).

The cellulite species population was not affected by the experimental diets, which was probably due to the same size of the silage particles size and the NDF and ADF in the experimental diets, and therefore the rate of passage of the silage particles was the same through the rumen (Sharifi *et al*. 2012).

The highest populations of Entodinium and total protozoa were observed in sheep fed the Sagunto, Cordona and 704 silage diets (P<0.05). The OM digestibility in these three diets was higher than in the other silage diets (Table 4). Entodiniums have the largest populations of protozoa and consume structural and non-structural carbohydrates (Pahlow and Zimmer, 1985; Williams and Coleman, 1988). Therefore, this type of protozoan had more growth and proliferation in the rumen, as increased the availability of these carbohydrates.

Table 3 The effect of type of silages in experimental diets on the intake and digestibility of nutrients in sheep

Item	Corn silage cultivars					SEM	P-value
	704	Dracma	Sagunto	Cordona	Lavida		
Nutrients intake (kg/day)							
Dry Matter	1.10	1.18	1.03	1.20	1.11	0.10	0.46
Organic matter	1.00	1.07	093	1.09	1.03	0.09	0.46
Crude protein	0.15	0.16	0.14	0.16	0.15	0.01	0.30
Eater Extract	0.02	0.02	0.02	0.02	0.02	0.002	0.06
NDF	0.43	0.45	0.39	0.45	0.41	0.04	0.45
ADF	0.26	0.27	0.26	0.27	0.22	0.02	0.28
NFC[1]	0.43	0.47	0.41	0.48	0.44	0.04	0.52
Nutrients digestibility (%)							
Dry Matter	72.8	68.8	75.3	70.7	67.9	1.74	0.06
Organic matter	76.5^{a}	72.7^{a}	79.1^{a}	75.1^{ab}	71.8^{b}	1.45	< 0.05
Crude protein	72.3	69.8	75.3	71.4	67.8	1.86	0.12
Eater Extract	83.9^{a}	70.9^{b}	81.6^{a}	72.7^{b}	77.9^{ab}	2.39	< 0.01
NDF[1]	64.2^{a}	58.3^{b}	66.4^{a}	60.1^{b}	54.0^{b}	1.86	< 0.01
ADF[2]	34.3^{a}	21.0^{b}	43.2^{a}	25.6^{b}	54.0^{b}	4.30	< 0.05
NFC[3]	91.2	88.9	93.4	91.5	88.7	1.36	0.16

[1] NFC= 100 – (ASH %+CP %+EE %+NDF %) (DePeters and Arosemena, 2000).
NDF: neutral detergent fiber; ADF: acid detergent fiber and NFC: non fiber carbohydrate.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 The effect of type of silages in experimental diets on ruminal parameters

Item	Corn silage cultivars					SEM	P-value
	704	Dracma	Sagunto	Cordona	Lavida		
Hours after feeding	The pH value of rumen fulid						
Zero	6.99	6.97	6.91	7.00	6.96	0.06	0.47
Two	6.44	6.48	6.47	6.48	6.46	0.11	0.99
Four	6.53	6.47	6.42	6.43	6.33	0.13	0.87
Six	6.38	6.39	6.44	6.43	6.33	0.09	0.94
Eight	6.13	6.28	6.28	6.33	6.39	0.13	0.94
Mean	6.55	6.54	6.56	6.55	6.55	0.05	0.99
Hours after feeding	NH_3-N concentration of rumen fluid of sheep (mg/dL)						
Zero	25.8	31.1	33.8	38.3	34.6	7.46	0.80
Two	27.8^{b}	25.5^{b}	38.5^{a}	22.6b^{b}	13.8^{c}	1.29	< 0.05
Four	15.8	12.7	6.61	25.4	12.7	4.28	0.16
Six	15.5	15.1	15.2	22.4	18.5	5.02	0.86
Eight	15.6	17.5	16.9	25.3	22.6	3.98	0.39
Mean	20.7	20.4	25.1	25.1	20.3	1.49	0.38
	Protozoan population of ruminal fluid ($\times 10^5$/mL of ruminal fluid)						
Holotrichs	0.91^{a}	0.79^{ab}	0.95^{a}	0.84^{a}	0.62^{b}	0.08	< 0.05
Cellulolytics	0.99	0.97	0.97	0.99	0.98	0.08	0.12
Entodiniums	27.5^{a}	23.4^{b}	28.2^{a}	26.6^{a}	23.7^{b}	1.41	< 0.05
Total protozoa	29.1^{ab}	25.2^{b}	30.2^{a}	30.6^{a}	25.5^{b}	1.44	< 0.05
	Daily excretion of purine derivatives in sheep urine (mmol/day) and microbial protein synthesis						
Allantoin	6.57	6.36	5.64	5.32	4.51	1.08	0.64
Uric acid	0.50	0.49	0.49	0.50	0.49	0.04	0.30
Xanthine and Hypoxanthine	0.92	0.91	0.82	0.77	0.67	0.23	0.99
Total purine derivatives	7.99	7.76	6.95	6.59	5.67	1.22	0.64
Microbial protein synthesis (g/day)	41.5	38.9	35.1	32.5	25.5	1.29	0.55
	Concentration of volatile fatty acids in ruminal fluid (mmol/L)						
Acetic acid	40.0	41.4	40.5	41.2	41.0	0.43	0.24
Propionic acid	13.0	12.9	12.6	12.9	13.0	0.25	0.80
Acetic acid to propionic ratio	3.09	3.21	3.20	3.19	3.15	0.07	0.77
Butyric acid	7.20	7.52	7.53	7.65	7.59	0.13	0.28
Valeric acid	0.22	0.49	0.51	0.31	0.31	0.18	0.70
Isovalric acid	0.78	0.78	0.71	0.85	0.83	0.10	0.87
Total fatty acids	61.2	63.1	62.0	62.9	62.7	0.61	0.25

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 The effect of silages in experimental diets on blood metabolites in sheep

Item	Corn silage cultivars					SEM	P-value
	704	Dracma	Sagunto	Cordona	Lavida		
Glucose (mg/dL)	82.1	85.6	87.6	90.3	90.3	4.97	0.77
Urea nitrogen (mg/dL)	15.5	15.6	17.9	16.3	16.2	1.46	0.67
Triglyceride (mg/dL)	28.0	31.0	26.0	25.3	32.6	7.04	0.86
Total protein (g/dL)	7.24	8.27	7.23	7.10	7.19	0.19	0.67

SEM: standard error of the means.

The total protozoa population in Sagunto, Cordona and 704 silages diets was higher ($P<0.05$), because the population of Entodinium protozoa was higher in the above three diets and Enthodinium had the highest number of protozoa.

There was not significant difference in purine base derivatives production in experimental diets (Table 4). Because, in the all experimental diets, the size of silage particles size and the chemical composition was the same, and there was a constant rate of degradation of carbohydrate and forage protein in the rumen. In this way, there was constant availability of fermentation energy and protein to bacteria (Yang and Beauchemin, 2007). On the other hand, there were constant NDF percentages and the level of concentrate in the diets, which was one of the reasons for the lack of significant differences in purine derivatives (Vakil Faraji *et al*. 2009). In one study, it was reported that the excretion of purine derivatives increased with increasing DM intake, while in the present experiment, DM intake was the same, which could be the reason for the lack of significant differences in purine excretion (Chen and Ørskov, 2004).

Microbial protein synthesis in the rumen was not affected by different silages in experimental diets. Because silages in the experimental diets did not affect the excretion of purine derivatives. In addition, CP, NDF of the diets and DM intake were the same, so the type of silages had no significant effect on microbial protein synthesis. Because the nature of carbohydrates such as soluble sugars, cellulose, hemicellulose or starch affects the production of microbial protein. If the cellulose and hemicellulose are increased in the diet, it will decrease the microbial protein entering the small intestine (Danesh Mesgaran *et al*. 2011).

The silages in experimental diets had not significant effect on ruminal VFA synthesis (Table 4). The ruminal concentration VFA`s in the rumen is affected by the types of feed, levels of DM intake, and fermentation levels in the rumen (Leonardi *et al*. 2005). In this experiment the ratio of forage: concentrate and DM intake were the same. In the experiment of Baldwin *et al*. (2004) the difference in OM intake was not significant enough to affect the molar concentration of total volatile fatty acids produced, acetic acid, propionic acid, butyric acid and the ratio of acetic acid to propionic acid.

Blood glucose concentrations were similar in sheep's due to the presence of similar molar ratios of rumen propionate (Table 5). When the intake of DM, OM, and CP in experimental diets were not significantly different, the production of ruminal volatile fatty acids would not be different, then blood metabolites would be the same. In ruminants, the main substrate for gluconeogenesis is ruminal propionate (Busquet *et al*. 2006).

In a study, Khosravi *et al*. (2018) reported that the lack of significant differences between blood parameters in cows was probably due to similar nutrient digestion and DM intake, which was in line with the present experiment. Normal sheep blood glucose concentration is 35-54.8 mg/100 mL (Radostits *et al*. 2016).

Blood urea nitrogen concentration indicates a balance between urea production in the liver and its excretion (Mojabi, 2011). Blood urea nitrogen concentration was the same between experimental animals. Blood urea nitrogen concentration has a positive correlation with ruminal NH_3-N concentration (Davidson *et al*. 2003), and in this experiment mean NH_3-N concentration was not affected by treatments.

In addition, the concentration of blood urea nitrogen is closely related to the CP concentration of the diet, and in the current experiment, the CP level of the diets was the same (da-Silva *et al*. 2018). The Triglyceride concentration was not affected by treatment, the lack of significant differences between treatments was probably due to the same ether extract intake (Roozbehan *et al*. 2014). Whole blood protein concentration was not affected by treatment and indicates the long-term protein status of dairy cows (Solaiman *et al*. 2010).

CONCLUSION

Lavida silage had the lowest ADF value, but the OM digestibility of diets in this silage were the lowest, probably due to more DM content, most of its starch was vitreous and there was more lignin in the stem. High lignin in the stem was identified with low digestibility of ADF in this silage diet. The diet with silage of Lavida cultivar had the lowest rumen NH_3-N concentration two hours after feed intake.

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