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Effects of malate supplementation to the concentrate feed on performance, rumen fermentation and carcass yield of lambs fed forage at restricted and ad-libitum level

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Summary: The effects of sodium and calcium salts of malic acid on growth performance, rumen pH, protozoa number, NH_3-N level and volatile fatty acid (VFA) ratio and carcass yield in Akkaraman lambs fed alfalfa hay either restricted or ad-libitum were investigated. In a completely randomized design experiment, treatments were arranged at 2x2 factorial fashion: two forage levels (100 g/d or ad-libitum) and two malate levels (0 or 5 g/d), which were tested in 32 male lambs (3-4 months of age, 23.28±1.27 kg BW). Adaptation to feeding lasted 10 days; while sampling period lasted for 60 days. Growth performance, feed intake, slaughter and carcass weight were not affected by the treatments. A significant change was found in ruminal pH by forage feding level over time. At the beginning of the experiment the amount of acetic acid was increased by ad-libitum alfalfa hay consumption and malate addition but this effect was disappeared end of the trial. Rumen propionic acid, NH_3-N concentration and protozoa number were not affected by the treatments but they changed by the sampling time. There was alfalfa hay level x malate supplementation x sampling time interaction effect on butyric acid concentration. In conclusion, malate addition did not improve growth rate and carcass yield of lambs but it affected ruminal acetic acid and butyric acid concentrations in lambs fed alfalfa ad-libitum.

Keywords: Acetic acid, alfalfa hay, carcass, malate, rumen fermentation.

Karma yemlere malat ilavesinin sınırlı ve serbest kaba yemle beslenen kuzularda performans, rumen fermantasyonu ve karkas randımanı üzerine etkileri

Özet: Araştırma malik asitin kalsiyum ve sodyum tuzunun sınırlı ve serbest kaba yem ile beslenen kuzularda büyüme performansı, rumen pH'sı, protozoa sayısı, amonyak azotu (NH_3-N) ve uçucu yağ asitleri miktarı ile karkas randımanı üzerine etkilerinin belirlenmesi amacıyla yürütülmüştür. Araştırmada 32 adet (23.28±1.27 kg canlı ağırlığında) 3-4 aylık yaşta erkek kuzular kullanılmış, çalışma tesadüf parsellerinde 2x2 faktöriyel (kaba yem, 100 g/gün veya ad-libitum; malat, 0 veya 5 g/gün) deneme deseninde yürütülmüştür. Araştırmada yeme alıştırma dönemi 10 gün ve deneme dönemi 60 gün olarak belirlenmiştir. Araştırma sonunda kuzularda büyüme performansı, yem tüketimi ile kesim ve karkas ağırlıkları bakımından gruplar arasında fark görülmemiştir. Rumen pH'sı bakımından kuru yonca otu x numune alma zamanı arasındaki interaksiyon önemli bulunmuştur. Araştırmanın başında alınan rumen sıvısı örneklerinde asetik asit miktarı, tüketilen ad-libitum kuru yonca otu ve malat ilavesi ile artmış, deneme sonunda bu etki ortadan kalkmıştır. Rumen propiyonik asit, NH_3-N miktarı ve protozoa sayısı muamelelerden etkilenmemiş, ancak numune alma zamanı bakımından değişiklik göstermiştir. Rumen sıvısında bütirik asit konsantrasyonu bakımından kuru yonca otu, malat ve numune alma zamanı arasındaki interaksiyon önemli bulunmuştur. Sonuç olarak yemlere malat ilavesinin kuzularda besi performansı ve karkas randımanını iyileştirmediği ancak rumende üretilen asetik ve bütirik asit miktarlarını etkilediği tespit edilmiştir.

Anahtar sözcükler: Asetik asit, karkas, malat, rumen fermentasyonu, yonca kuru otu.

Introduction

Efficient animal production requires good management practices such as appropriate feed formulation and feeding programs, animals' health care and hygiene. Digestive disorders are one of the main causes that lead to deteriorations in livestock performance and production efficiency. In ruminants, prevention of the digestive disorders which require normalization of ruminal pH and microbial flora is the most crucial factor for effective and profitable animal production. Most of nutrients supplied to ruminants are produced in the rumen from microbial metabolism of feeds. Feeds and appropriate feeding strategies are essential while feed additives can help stabilize the ruminal environment. A steady rumen environment is crucial for the health and performance of ruminant animals (38).

Feed additives are not a warranty for animals' health, high production or productivity; while they are important for the improvement and maintenance of rumen fermentation, general health, growth, reproduction and animal products quality (28). As public concern over the use of antimicrobials in animal nutrition increased in the years following the approval of ionophores as feed additives, research efforts concentrated on the development of alternative types of feed additives such as enzymes, probiotics and organic acids. The use of organic acids as feed additives received significant importance in the early 2000s due to the ban of antibiotics within the European Union. These weak carboxylic compounds are commonly found in biological tissues and have been used in animal production for decades. Malic acid is found in forages in ruminant diets'. Although *in vitro* researches have shown favourable effects of malate salts on ruminal fermentation through increased ruminal pH and propionate production (24, 25, 33, 37), the effects of malic acid and malate on *in vivo* ruminal fermentation and the performance of ruminant are limited and inconsistent (6). Hence, more in vivo research is required to give a reliable conclusion. The effects of malic acid supplementation on animal performance and ruminal fermentation can be based on many mechanism of action like composition of diets, type, amount and quality of forage, forage:concentrate ratio of diet or malate dose used in diets (7). There are no data about the effect of malate with different forage levels on the growth performance and rumen pH, NH_3-N level and volatile fatty acid (VFA) ratio in lambs. Therefore, the purpose of this research was to examine the influence of different alfalfa hay consumption level and malate addition on performance, carcass criteria and some rumen fermentation pattern.

Materials and methods

Lambs, feeds and experimental procedures: This study was conducted between May-August 2015 in the Education and Research Farm of Ankara University (Ethical Approval Protocol No: 2014-13-76). During the experiment, minimum and maximum ambient temperature ranged from 20 to 35°C. A total of 32 male Akkaraman lambs (fat tailed, 3-4 months old, 23.28±1.27 kg BW), were used. Animals were housed in individual cages (0.90 x 1.15 m) and fed a basal diet typically offered to growing lambs in Middle Anatolia (Table 1) which is formulated to provide adequate nutrients for growing lambs (26). Basal diet consisted mostly of barley, triticale, corn and sunflower meal plus other ingredients in ratios dependent to the growing period. Dietary treatments in the current study were, 1) limited alfalfa hay without malate, LC; 2) limited alfalfa hay with 5g/d malate, LM; 3) ad-libitum alfalfa hay without malate, AC; and 4) ad-libitum alfalfa hay with 5g/d malate, AM. Malate concentration was selected according to the manufacturer's recommendations for sheep. Malate was mixed with the concentrate at the time of feeding. Feed was provided twice daily at 8:30 a.m. / 6:30 p.m and diets and water were provided ad libitum in experimental groups except alfalfa hay in LC and LM. In restricted forage groups (LC and LM), lambs were allowed only 100 g/day alfalfa hay in two equal meal. All lambs were vaccinated for enterotoxemia, endo- and exoparasites before the experiment.

Sampling and analytical methods: Samples of the concentrate feed mix and alfalfa hay were collected during the experimental period for chemical composition analyses. Dry matter, crude protein, crude fiber, crude ash and crude fat in alfalfa hay and concentrate feed mix were analysed according to the methods in AOAC Official Methods (1). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured according to the methods described by Van Soest et al. (35) using an ANKOM^{200} Fiber Analyzer (ANKOM Technology Corp., Fairport, USA). NDF, using sodium sulphite, heat stable amylase, and ADF were represented including residual ash. Metabolisable energy of forage and concentrate mix were calculated according to TSE (34). The nutrient composition of the concentrate feed mix and alfalfa hay are shown in Table 1.

The experiment initiated with an adaptation period (10 day) which was followed by a 60 day period while body weight changes and feed consumption were recorded. Lambs were individually weighted weekly before feeding time and body weight gains were calculated by subtracting the previous weight from the next week weight. Concentrate feed mix and alfalfa hay orts by animal's cage were taken every day and weighted weekly. Feed conversion ratio (FCR) was calculated as (kg feed consumed)/(kg weight gained).

Table 1. Nutrient composition of concentrate feed and alfalfa hay
Tablo 1. Kuru yonca otu ve konsantre yemin besin maddesi bileşimi

Feeds	DM[1]	CP[1]	Ash[1]	CF[1]	EE[1]	NDF[1]	ADF[1]	ME[2]
Grower Feed	90.89	18.00	7.77	9.03	3.55	25.94	12.44	2631.41
Alfalfa Hay	92.35	13.12	8.29	33.99	-	49.01	8.09	2008.84

[1]results of chemical analyses
[2]calculated according to the TSE

On days 10 and 56, rumen contents were collected from 5 lambs in each treatment throughout a rumen catheter. The rumen fluid (~50 ml) was collected at 0, 2, 4 and 6 hours after feeding. The pH values were immediately measured using a pH-meter (Ion 6, Acornseries, Oakton).

Rumen fluid samples were transported immediately to the laboratory in an ice bucket, 1 ml of sample was carefully mixed with 1 ml of a solution of 0.6 g methylgreen, 6 g NaCl and 100 ml formaldehyde (37%) filled up to 1000 ml aqua dest for protozoa counting. Then, parts of the samples were transferred into a Fuchs-Rosenthal counting chamber (0.0625 mm^2; 0.2 mm deep; Marienfeld, Germany). Total numbers of protozoa were determined using a light microscope (Leica CME). Also some rumen liquid samples were frozen at –20 ºC for NH_3-N and VFA concentration determination.

Ruminal fluid samples for VFA analysis were melted at +4 ºC and centrifuged at 13000 g for 30 min at 4 °C. VFAs were determined by the method of Oeztuerk et al. (27) using HPLC (Thermo Seperation Product-Spectra Product P1000 (USA)) with a Rezex ROA-organic acid column (7.8 × 300 mm, Phenomenex) and Rezex ROA-organic acid column (50x7.8 mm, Phenomenex) (at 60 °C, isocraticelution with 0.005 M H2SO4 and UV detection at 210 nm. NH_3-N concentration was detected using a spectrofotometric method as reported by Chaney and Marbaeh (8).

Statistical Analysis: Differences between groups were analysed using the general linear models (GLM) procedure of SAS (31). A repeated measures ANOVA was conducted with time for rumen parameters. The statistical model included malate, forage, time (sampling time), malate × forage, malate × time, forage × time and malate x forage x time interactions. Results were represented as means ± standard error of means (SEM). A 2x2 factorial arrangement was adopted to evaluate the forage and malate effects. The statistical model included main effects of forage level and malate supplementation and their interaction. Differences were considered significant if $P < 0.05$.

Results

Feed intake, body parameters and carcass ratio: The effects of the addition of malic acid salts to a basal diet on lamb performance are presented in Table 2. The initial and final BW did not differ between the experimental groups (P>0.05). BW gain, feed intake and carcass parameters did not affect by treatments. The final body weight and carcass weight in LC, LM, AC and AM groups were 38.20, 39.05, 39.62, 39.85; 14.80, 15.20, 15.80 and 16.17 kg, respectively. Feed conversion ratio was also not influenced by dietary treatment (P>0.05) (Table 2).

Ruminal pH, NH_3-N concentration and protozoa number: Results of ruminal pH, concentration of NH_3-N and protozoa number are shown in Table 3, 4 and 5. Ruminal pH changed from 6.98 to 7.19 (on day 10th), 7.13 to 7.22 (on day 56th) before the feeding time.

An alfalfa hay consumption × time of sampling interaction was detected for pH on day 10^{th} (P = 0.018). An afalfa hay consumption × time of sampling interaction was detected for pH on day 10th (P = 0.018). The ruminal pH decreased after feed consumption by lambs. Meanwhile, at the end of the study (day 56) this effect did not occur (P=0.081)(Table 3). There was no significant difference in rumen NH_3-N and protozoa number between experimental groups on day 10^{th} or 56^{th} (Table 4 and 5). Rumen fermentation patterns start to change immediately after feeding concentrate feed mix. In this study, there was a gradually decrease in pH and protozoa numbers whereas an increase in NH_3-N concentration after feed consumption.

Table 2. Effects of malate addition on performance and carcass yield by restricted and ad-libitum forage fed lambs
Tablo 2. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin performans ve karkas randımanı üzerine etkileri

	Restricted Hay		Ad-libitum Hay		P -Value		
Variable	Malate	Malate	Malate	Malate	FL	M	FLxM
	(-)	(+)	(-)	(+)			
Initial BW	22.80±1.31	22.82±3.05	23.72±1.54	23.68±1.43	0.621	0.997	0.986
Final BW (kg)	38.20±1.89	39.05±3.87	39.62±3.24	39.85±3.13	0.718	0.860	0.919
BWG (kg)	0.275±0.03	0.290±0.02	0.284±0.03	0.289±0.04	0.897	0.756	0.871
SW (kg)	38.70±1.78	39.65±3.91	40.72±3.15	41.38±3.47	0.554	0.798	0.964
CW (kg)	17.82±0.53	18.14±1.90	18.87±1.51	19.50±1.27	0.458	0.771	0.990
CR (%)	46.04±0.78	45.76±1.32	46.35±1.39	47.13±1.20	0.561	0.862	0.711
CFI (g/d)	1476±79.50	1440±152.00	1573±118.00	1519±123.00	0.423	0.607	0.820
FCR	5.34±0.28	4.94±0.35	5.41±0.24	5.45±0.36	0.346	0.641	0.461
MFC (g/d)	100	100	140.3±13.40	174.0±27.00	NA	0.286	NA

FL: Forage level; M: Malate; FLxM: Forage level x malate interaction; BW: Body weight; BWG: Body weight gain; SW: Slaughter weight; CW: Carcass weight; CR: Carcass ratio; CFI: Concentrate feed intake; FCR: Feed conversation ratio; MFC: Mean forage consumption; NA: Not applicable

Table 3. Effects of malate addition on ruminal pH by restricted and ad-libitum forage fed lambs
Tablo 3. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin rumen pH'sı üzerine etkileri

		Ph (day 10th)				Ph (day 56th)			
Treatments		***Hours***							
		0	2	4	6	0	2	4	6
Restricted Hay	Malate(-)	6.98±0.10	6.02±0.18	6.30±0.10	5.93±0.14	7.22±0.11	6.10±0.09	5.59±0.05	5.69±0.21
	Malate(+)	7.11±0.11	6.34±0.19	6.28±0.20	6.22±0.26	7.21±0.08	5.92±0.19	5.76±0.11	5.70±0.12
Ad-libitum Hay	Malate(-)	7.17±0.07	6.02±0.16	5.98±0.18	6.06±0.16	7.15±0.12	5.90±0.09	5.98±0.17	5.93±0.14
	Malate(+)	7.19±0.05	6.02±0.06	5.83±0.11	5.71±0.13	7.13±0.07	6.19±0.10	5.96±0.12	5.98±0.06
Main effects									
Forage feeding level									
Restricted		7.05±0.07a	6.18±0.14b	6.29±0.11b	6.08±0.15b	7.21±0.06	6.01±0.11	5.68±0.06	5.70±0.12
Ad-libitum		7.18±0.04a	6.02±0.08b	5.91±0.10b	5.88±0.11b	7.14±0.06	6.05±0.08	5.97±0.10	5.96±0.07
Malate									
(-)		7.08±0.07	6.02±0.11	6.14±0.11	5.99±0.10	7.19±0.08	6.00±0.07	5.79±0.11	5.81±0.13
(+)		7.15±0.06	6.18±0.11	6.06±0.13	5.97±0.16	7.17±0.05	6.05±0.11	5.86±0.08	5.84±0.08
P Values									
Time		0.000				0.000			
Malate		0.788				0.664			
FL		0.199				0.094			
FLxM		0.211				0.597			
FLxT		0.018				0.081			
MxT		0.427				0.958			
FLxMxT		0.315				0.233			

FL: Forage level; M: Malate; FLxM: Forage level x malate supplementation interaction; FLxT: Forage level x time interaction; MxT: Malate supplementation x time interaction; FLxMxT: Forage level x malate supplementation x time interaction; a-b; P<0.05

Table 4. Effects of malate addition on ruminal concentration by restricted and ad-libitum forage fed lambs
Tablo 4. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin rumen NH_3-N konsantrasyonu üzerine etkileri

		NH_3-N (mmol/l) (day 10th)				NH_3-N (mmol/l) (day 56th)			
Treatments		Hours							
		0	2	4	6	0	2	4	6
Restricted Hay	Malate(-)	12.87±1.47	19.49±1.99	17.35±2.62	17.18±3.17	9.38±1.17	24.97±2.10	23.63±1.51	20.35±1.19
	Malate(+)	11.23±1.44	14.10±3.26	11.09±2.82	12.33±3.33	11.02±1.65	22.04±0.99	21.75±2.91	22.45±5.05
Ad-libitum Hay	Malate(-)	10.28±1.34	17.23±2.64	14.93±2.32	14.74±349	9.54±0.99	25.32±1.92	20.31±2.44	20.65±3.35
	Malate(+)	9.84±1.31	14.92±2.57	15.14±0.90	14.27±1.18	10.22±1.68	21.13±2.74	21.58±2.25	19.73±1.83
Main effects									
Forage level									
Restricted		12.05±0.01	16.80±2.01	14.22±2.09	14.75±2.31	10.20±0.99	23.51±1.20	22.69±1.58	21.40±2.47
Ad-libitum		10.06±0.89	16.07±1.78	15.03±1.17	14.50±1.74	9.88±0.93	23.22±1.72	20.95±1.58	20.19±1.81
Malate									
(-)		11.57±1.03	18.36±1.60	16.14±1.70	15.96±2.26	9.46±0.72	25.14±1.34	21.97±1.46	20.50±1.68
(+)		10.53±0.95	14.51±1.96	13.11±1.55	13.30±1.69	10.62±1.12	21.58±1.38	21.67±1.74	21.09±2.57
P Values									
Time		0.000				0.000			
Malate		0.224				0.759			
FL		0.800				0.606			
FLxM		0.379				0.878			
FLxT		0.528				0.932			
MxT		0.518				0.294			
FLxMxT		0.570				0.692			

FL: Forage level; M: Malate; FLxM: Forage level x malate supplementation interaction; FLxT: Forage level x time interaction; MxT: Malate supplementation x time interaction; FLxMxT: Forage level x malate supplementation x time interaction

Table 5. Effects of malate addition on ruminal protozoa number by restricted and ad-libitum forage fed lambs
Tablo 5. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin rumen protozoa sayısı üzerine etkileri

		Protozoa number (10^4) (day 10^{th})				Protozoa number (10^4) (day 56^{th})			
Treatments		Hours							
		0	2	4	6	0	2	4	6
Restricted Hay	Malate(-)	176±67.01	108±44.71	50±16.35	186±71.54	122±30.60	43±6.04	40±13.78	53±13.09
	Malate(+)	138±62.78	62±15.78	73±22.62	87±39.96	135±29.66	61±11.45	61±17.28	50±15.17
Ad-libitum Hay	Malate(-)	105±54.31	55±11.40	40±12.15	81±23.84	128±39.80	73±40.02	74±29.93	69±28.96
	Malate(+)	148±51.10	77±32.00	100±25.59	78±25.91	167±17.29	85±25.45	45±10.00	35±8.06
Main effects									
Forage level									
Restricted		157±43.75	85±23.62	61±13.70	136±42.00	128±20.20	52±6.79	50±10.99	51±9.45
Ad-libitum		126±35.88	66±16.42	70±16.68	79±16.60	147±21.46	79±22.44	59±15.64	62±14.36
Malate									
(-)		140±42.35	81±23.48	45±9.75	133±39.62	125±23.68	58±19.72	57±16.53	61±15.21
(+)		143±38.19	69±17.00	86±16.72	82±22.50	151±17.04	73±13.75	53±9.78	52±8.14
P Values									
Time		0.002				0.000			
Malate		0.885				0.715			
FL		0.461				0.407			
FLxM		0.293				0.793			
FLxT		0.459				0.842			
MxT		0.171				0.382			
FLxMxT		0.904				0.416			

FL: Forage level; M: Malate; FLxM: Forage level x malate supplementation interaction; FLxT: Forage level x time interaction; MxT: Malate supplementation x time interaction; FLxMxT: Forage level x malate supplementation x time interaction

Table 6. Effects of malate addition on rumen acetic acid concentration by restricted and ad-libitum forage fed lambs
Tablo 6. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin rumen asetik asit konsantrasyonu üzerine etkileri

		Acetic acid (mmol/l) (day 10^{th})				Acetic acid (mmol/l) (day 56^{th})			
Treatments		Hours							
		0	2	4	6	0	2	4	6
Restricted Hay	Malate(-)	25.26±4.78	32.11±7.63	50.70±11.30	40.77±2.86	23.98±8.06	29.61±6.65	44.01±4.95	45.58±4.56
	Malate(+)	38.60±13.10	62.32±8.62	38.26±4.67	50.70±10.90	34.80±15.30	36.01±8.28	61.88±8.59	65.36±9.30
Ad-libitum Hay	Malate(-)	35.27±7.67	52.20±10.70	41.74±2.18	45.71±5.81	27.23±7.58	46.66±7.22	37.72±8.50	53.40±10.40
	Malate(+)	73.82±7.10	60.50±10.50	62.34±9.18	58.43±2.90	43.6±10.20	48.65±6.92	36.50±15.60	65.01±8.32
Main effects									
Forage level									
Restricted		31.61±6.93B	47.21±7.40B	44.46±6.12B	45.76±5.57B	29.38±8.34	32.81±5.12	52.95±5.54	55.47±5.89
Ad-libitum		54.54±8.10A	56.31±7.21A	52.04±5.62A	52.07±3.72A	35.43±6.58	47.66±4.73	37.10±8.36	59.22±6.57
Malate									
(-)		30.27±4.58B	42.13±7.04B	46.20±5.62B	43.24±3.16B	25.61±5.24	38.14±5.43	40.86±4.75	49.51±5.51
(+)		56.19±9.15A	61.39±6.41A	50.30±6.30A	54.58±5.48A	39.20±8.78	42.33±5.51	49.18±9.38	65.18±5.88
P Values									
Time		0.563				0.001			
Malate		0.000				0.090			
FL		0.004				0.708			
FLxM		0.176				0.581			
FLxT		0.509				0.078			
MxT		0.310				0.762			
FLxMxT		0.115				0.771			

FL: Forage level; M: Malate; FLxM: Forage level x malate supplementation interaction; FLxT: Forage level x time interaction; MxT: Malate supplementation x time interaction; FLxMxT: Forage level x malate supplementation x time interaction; A-B; $P<0.01$

Table 7. Effects of malate addition on rumen propionic acid concentration by restricted and ad-libitum forage fed lambs
Tablo 7. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin rumen propiyonik asit konsantrasyonu üzerine etkileri

		Propionic acid (mmol/l) (day 10th)				Propionic acid (mmol/l) (day 56th)			
Treatments		Hours							
		0	2	4	6	0	2	4	6
Restricted Hay	Malate(-)	17.77±7.02	22.17±4.46	21.88±2.39	22.55±1.28	5.85±1.46	26.81±8.26	46.18±5.50	37.50±5.30
	Malate(+)	18.04±7.83	34.97±4.78	15.12±2.75	30.33±9.67	9.50±3.29	10.11±1.97	34.10±5.16	41.67±8.58
Ad-libitum Hay	Malate(-)	40.10±15.50	27.07±7.13	24.72±5.15	38.80±10.90	10.71±3.45	37.67±7.12	37.40±10.10	37.65±5.21
	Malate(+)	10.94±3.17	30.82±2.24	30.34±8.46	32.88±5.51	9.44±1.04	34.66±6.54	29.60±7.49	39.90±6.12
Main effects									
Forage level									
Restricted		17.91±4.96	28.57±3.75	18.50±2.05	26.44±4.78	7.67±1.80	23.46±4.16	40.14±4.09	39.59±4.81
Ad-libitum		25.54±8.91	28.95±3.58	27.53±4.76	35.83±5.82	10.07±1.71	36.16±4.59	33.48±6.06	38.77±3.81
Malate									
(-)		28.95±8.85	24.62±4.05	23.30±2.72	30.66±5.82	8.28±1.94	32.24±5.45	41.77±5.61	37.58±3.50
(+)		14.49±4.15	32.90±2.58	22.73±4.90	31.61±5.26	9.47±1.62	27.38±4.03	31.85±4.35	40.78±4.98
P Values									
Time		0.135				0.000			
Malate		0.745				0.434			
FL		0.153				0.563			
FLxM		0.275				0.964			
FLxT		0.736				0.134			
MxT		0.107				0.378			
FLxMxT		0.165				0.931			

FL: Forage level; M: Malate; FLxM: Forage level x malate supplementation interaction; FLxT: Forage level x time interaction; MxT: Malate supplementation x time interaction; FLxMxT: Forage level x malate supplementation x time interaction

Table 8. Effects of malate addition on rumen butyric acid concentration by restricted and ad-libitum forage fed lambs
Tablo 8. Sınırlı ve serbest kaba yemle beslenen kuzularda malat ilavesinin rumen bütirik asit konsantrasyonu üzerine etkileri

		Butyric acid (mmol/l) (day 10th)				Butyric acid (mmol/l) (day 56th)			
Treatments		Hours							
		0	2	4	6	0	2	4	6
Restricted Hay	Malate(-)	8.59±3.15	11.27±2.29	10.18±2.38	10.71±2.78	12.29±3.91	6.08±1.75	10.62±1.46	8.98±1.95
	Malate(+)	6.35±1.32	9.86±2.47	8.81±2.59	16.59±1.93	5.66±2.72	11.64±3.18	24.78±6.98	12.77±2.02
Ad-libitum Hay	Malate(-)	7.53±2.51	8.28±3.08	10.14±3.51	10.99±4.02	2.07±0.09	9.47±4.69	11.45±2.84	11.74±1.48
	Malate(+)	5.81±2.18	11.56±1.87	11.61±2.77	16.04±2.21	1.68±0.44	10.96±2.23	4.76±1.27	16.04±3.60
Main effects									
Forage level									
Restricted		7.47±1.65	10.56±1.60	9.50±1.68	13.65±1.87	8.97±2.50	8.86±1.95	17.70±4.11	10.88±1.47
Ad-libitum		6.67±1.59	9.92±1.78	10.87±2.12	13.52±2.32	1.85±0.25	10.32±2.16	7.62±1.86	14.19±2.19
Malate									
(-)		8.06±1.91	9.78±1.88	10.16±2.00	10.85±2.30	8.46±2.99	7.35±1.96	10.93±1.29	10.02±1.36
(+)		6.08±1.21	10.71±1.49	10.21±1.85	16.32±1.39	3.89±1.60	11.34±1.91	15.88±5.12	14.22±1.90
P Values									
Time		0.000				0.005			
Malate		0.577				0.335			
FL		0.980				0.138			
FLxM		0.651				0.265			
FLxT		0.872				0.014			
MxT		0.084				0.261			
FLxMxT		0.785				0.023			

FL: Forage level; M: Malate; FLxM: Forage level x malate supplementation interaction; FLxT: Forage level x time interaction; MxT: Malate supplementation x time interaction; FLxMxT: Forage level x malate supplementation x time interaction

Rumen volatile fatty acid concentration: The effect of malate supplementation on ruminal acetic, propionic and butyric acid concentration are presented in Table 6, 7 and 8 (10^{th} day and 56^{th} day).

The amount of alfalfa consumption and malate addition in lambs' diet caused significant ($P<0.01$ and $P<0.001$, respectively) differences in ruminal acetic acid concentration (Table 6). The malate supplementation and ad-libitum alfalfa hay intake increased ruminal acetic acid concentration in day 10^{th}. In our study, malate supplementation under the experimental conditions had no effects on rumen propionic acid concentration (Table 7). There was an alfalfa consumption x malate x time of sampling interaction for ruminal butyric acid content on day 56^{th}($P = 0.023$)(Table 8).

Discussion and Conclusion

Performance, feed intake and carcass weight: In the current study, malate supplementation in concentrate feeds did not effect of fattening lambs performance. Previous studies showed no effects of malate supplementation on performance of cattle (3) and lambs (18,22). Likewise, malate supplementation of growing lambs diet at the level of 4 or 8 g/kg of concentrate feed did not affect FCR or lambs' growth performance (6). There were no differences in feed intake (Table 2), which agrees with previously reported results (20,21). On the other hand, in bull calves, malate addition improved average daily gain (ADG) and FCR (31). Similarly Flores (10) claimed that malate addition enhanced performance and digestibility of nutrients in intensively fattened lambs. DL-malate treatment in finishing beef cattle increased feed efficiency and ADG by 21 and 22%, respectively, during the first 10 days dietary adaptation period and the authors attributed these results to a reduction in subclinical acidosis (20). In the current study, neither carcass weight nor carcass yield did not change among the treatments. In agreement with these observations in the study with higher levels DL-malate supplementation in high forage diet had no effect on carcass characteristics in cattle (20), and in heifers (4). The lack of a positive effect of malate supplementation in our study could be attributed to alfalfa hay because of its' malic acid content. Similarly, Callaway et al. (2) reported that some of the benefits associated with alfalfa in the diets of ruminants may be due to the malate in that forage. Salama et al. (29) declared that malic acid supplementation of dairy goat diets did not increase milk production because of the high concentration of malic acid in the basal diet which contain high proportion of alfalfa. Likewise, it may be due to malic acid content of the basal diet or the amount of malate supplemented in the experimental diets; meanwhile since feedstuffs and concentrate feeds were not assayed for malic acid, it can not be confirmed.

Rumen fermentation: Based on our results, rumen pH at 0 and 2, 4 and 6 h post-feeding were unchanged by experimental procedures and the values changed at 5.59 to 6.34 after feeding time. These values were slightly lower than the normal range (6.0 - 7.0) which was reported by Hoover (13) for optimal rumen fermentation. In the present study, concentrate feed ratio approached 93% in feed basis. Fortunately, lambs fed medium quality hay (13.12% CP and 49.01 NDF, as feed basis). It is well known that fattening systems of lambs' need high-concentrate diets which may lead to depressed pH. In the rumen, excessive amounts of rapidly fermentable carbohydrates decrease ruminal pH.

Addition of carboxylic acid salts stimulate the transformation of lactic acid into propionic acid throughout the *Selenomanas ruminantium* by using the succinate-propionate pathway (12). Therefore, malate might perform to increase the pH of rumen. Malate might stimulate propionate production in rumen because it is a key intermediate in the production of succinate or propionate (5). Malate addition was found to increase the ruminal pH, propionate and butyrate production and decreased acetate:propionate ratio *in vitro* (19, 23) and in dairy steers (15). Malic acid supplementation in steers' diet increased ruminal pH 2 h after feeding in the study of Montana et al. (21). Likewise Sahoo and Jena (28) dedicated that supplementing ruminant diets with malic acid or its salts are found to be effective in reducing the decline in ruminal pH shortly after feeding. However, in the current study, neither malate addition nor alfalfa consumption level did not change the ruminal pH in any sampling time. Khampa et al. (14) and Malekkhahi et al. (18) also reported that malate addition did not affect ruminal pH and ammonia concentrations in lactating dairy cows and lambs, respectively. Similar to our results, Vyas et al. (36) did not observe important effect on ruminal pH and fermentation parameters when malate addition in high-grain feedlot steers' diet. The reason for the difference among research results about ruminal pH is unclear, but in our study ruminal liquid samples were collected by stomach tube which might have influenced the pH values. The other reason may be related to *in vivo* and *in vitro* systems because they have different experimental conditions. For instance, *in vivo* systems are much more complex and intensive in terms of microbial concentrations. Another reason for the incoherence between the studies could be differences in the feeding systems. In the present study, malate were supplemented on the concentrate feed mix, whereas malate was infused in to the rumen 30 minute after the morning feeding in Martin et al. (20)'s study. Moreover, greater levels of

malate used in *in vitro* studies may be induce ruminal fermentation and to enhance animal performance.

Our data indicate that under the experimental conditions, malate addition had no effect on NH_3-N concentration and protozoa number. Similarly, Devant et al. (9), Malekkhahi et al. (18) and Sniffen et al. (32), observed no change in NH_3-N concentration. In contrast to our results, Carrasco et al. (3) showed that malate indicated higher ruminal NH_3-N concentrations in heifers. Conversely, Liu et al. (17) found ruminal pH and NH_3-N concentration reduced in malic acid supplementation in steers.

In the current study, the use of malate and ad-libitum alfalfa hay consumption increased ruminal acetic acid concentration (Table 4) but no difference was observed in propionic acid and butyric acid concentration except for butyric acid concentration on day 56^{th}. Interaction (alfalfa hay x malate x sampling time) was observed in butryric acid concentration (Table 6). In the Martin et al. (20)'s study with steers (485 ± 24.8 kg) were fed rolled grain (80%) and DL-malate was into the rumen on two sequential days (0-80 g of DL-malate/d). At the end of their research, consistent with our findings they informed that an increase ruminal acetic acid concentration without changes in propionic and butyric acid proportions. As different from our results in early-lactation dairy cows, malate addition increased acetate, butyrate, and total VFA concentration (16). Malate treatment increased molar proportion of butyrate in rumen but the other ruminal VFAs did not affect (6). Conversely, Foley et al. (11) and Malekkhahi et al. (18) showed that no effects were found between groups with respect to acetate and butyrate molar proportions when adding malate increased the molar proportions of propionate. In another study, malate addition did not affect propionic acid and butyric acid concentration in dairy cows (9). Similarly, in the studies of Montano et al. (21), malate did not change acetate, propionate, and butyrate concentration in the rumen. In these different results could be related to the different experimental conditions. The reason for the increased acetate may be due to malate is fermented by ruminal microorganisms to acetate and propionate but the reason for the butyrate is unclear.

In summary malate supplementation and ad-libitum alfalfa hay consumption did not affect the BWG, FCR and carcass ratio of Akkaraman lambs. An increase in acetic acid concentration was found along with an interaction in terms of ruminal butyric acid concentration and hay consumption rate. Since there are many contradictory studies in the field, more research under different feeding regimes is necessary to clarify the effects (especially on rumen fermentation) of malate supplementation to the ruminant diets.

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