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The Effectiveness of DL-Malic Acid on Fattening Performance and Rumen Parameters in Beef Cattle Rations Containing High Concentrated Feed and Dry Forage*

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Abstract: This study aimed to determine the effects of DL-malic acid supplementation in beef cattle rations containing high concentrated feed and dry forage (alfalfa hay and wheat straw) on fattening performance, carcass traits and rumen parameters. In present study, 47 male beef cattle (19 Simmental, 28 Limousine) at the age of 14-16 months were used. Cattle breeds were divided into two groups (0 and 30 g/day of DL-malic acid). In the present study, the differences in live weights, daily live weight gains and carcass weights of the control and DL-malic acid groups were found to be significant in Simmental breed (P<0.05), but insignificant in Limousine breed. The beef cattle body measurements (withers height, chest girth, rump height, body length) did not change with malic acid supplementation (P>0.05).The malic acid addition increased the molarities of acetic, butyric, propionic and total volatile fatty acids (TVFA) and acetic acid/ propionic acid ratio in the rumen fluid (P<0.05).The addition of malic acid had no significant effect on the number of ciliated protozoa (*Entodinium*, *Diplodinium*, *Isotricha* and *Dasytricha*) and total bacteria count in the rumen fluid (P>0.05). As a result, DL-malic acid supplementation to beef cattle ration varied with cattle breeds in terms of fattening performance, while such supplementations had positive effect on fattening in Simmental breed. Besides, the increase in TVFA and acetic, propionic and butyric acids, which are indicators of fiber and carbohydrate fermentation efficiency in the rumen, shows that malic acid has a positive effect on feed digestion in the rumen.
Keywords: DL-malic acid, Limousine, fattening performance, rumen parameters, Simmental

Yüksek Düzeyde Konsantre Yem ve Kuru Kaba Yem İçeren Besi Sığırı Rasyonlarında DL-Malik Asitin Besi Performansı ve Rumen Parametreleri Üzerine Etkinliği

Öz: Bu çalışmada, yüksek konsantre yem ve kuru kaba yem (yonca otu ve buğday samanı) içeren besi sığırı rasyonlarına DL-malik asit ilavesinin besi performansı, karkas özellikleri ve rumen parametreleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Bu çalışmada 14-16 aylık yaştaki 47 erkek besi sığırı (19 Simental, 28 Limousine) kullanıldı. Sığır ırkları iki gruba ayrıldı (0 ve 30 g/gün DL-malik asit). Kontrol ve DL-malik asit gruplarının canlı ağırlıkları, günlük canlı ağırlık artışları ve karkas ağırlıkları arasındaki farklılık Simmental ırkında önemli (P<0.05), Limousine ırkında ise önemsiz bulundu. Besi sığırlarının vücut ölçüleri (cidago yüksekliği, göğüs çevresi, sağrı yüksekliği, vücut uzunluğu) malik asit takviyesi ile değişmedi (P>0.05). Malik asit ilavesi rumen sıvısındaki asetik, bütirik, propiyonik ve toplam uçucu yağ asitlerinin (TVFA) molaritelerini ve asetik asit/propiyonik asit oranını arttırdı (P<0.05). Malik asit ilavesinin rumen sıvısındaki silliatalı protozoa (*Entodinium, Diplodinium, Isotricha* ve *Dasytricha*) sayısı ve toplam bakteri sayısı üzerinde önemli bir etkisi olmadı (P>0.05). Sonuç olarak, besi sığırı rasyonlarına DL-malik asit takviyesinin besi performansı açısından sığır ırklarına göre farklılık gösterdiği ve Simental ırkında besiye olumlu etkisinin olduğu belirlendi. Ayrıca rumende lif ve karbonhidrat fermentasyon etkinliğinin göstergesi olan TVFA ile asetik, propiyonik ve bütirik asitlerdeki artış dikkate alındığında, malik asidin rumende yem sindirimine etkisinin olumlu olduğu söylenebilir.
Anahtar kelimeler: Besi performansı,DL-malik asit, Limuzin, rumen parametreleri,Simental

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Introduction

Organic acids are either naturally encountered in various plant and animal substrates or generated through biochemical metabolism of these substrates and they have recently been used as alternative feed additives in animal rations (Ricke, 2003; Dittoe et al., 2018). Organic acids have long been used as food additives and preservatives, but they don't have much history for use in animal nutrition (Sahoo and Jena, 2014). It was reported that organic acid supplementations to animal diets reduced feed pH, brough in a positive aroma to animal feeds, increased feed consumption, increased secretion of digestive enzymes secreted from pancreas, preserved electrolyte balance in gastro-intestinal system and increased digestion and absorption of the minerals (Dibner and Buttin, 2002). Naturally occurring or fermentation-induced organic acids, like lactic acid, citric acid, malic acid, are the compounds generally composed of fatty acids included in carboxyl group (-COOH) and salt forms like Ca-formate, Ca-propionate. In compound feed industry, to prevent heat-induced destruction of organic acids especially during the pelletizing phase and to improve efficiency of organic acids, less odorous and less volatile salt forms are supplemented into compounds instead of natural organic acids. Strong or weak effects of organic acids may vary based on acid chain length, degree of saturation, ambient microflora (rumen or intestine), type of bacteria-like chemical and environmental factors (Mirza et al., 2016; Dittoe et al., 2018). High doses of organic acids can have a negative effect on rumen fermentation. Kara et al. (2015) determined that high doses formic acid increased in vitro enteric methane production and low doses decreased in vitro total gas production, metabolic energy and organic matter digestion and high doses have increased all these parameters of alfalfa herbage.

Malic acid is used as nutrient by the bacteria group converting lactic acid into propionic acid, a primary material of glucose production, through stimulating lactate use by *Selenomonas ruminantium*. Malic acid supplementation into ruminant rations reduces rate of decrease in post-feeding rumen pH. Additionally, malic acid-containing diets reduce number of protozoa and methane release (Kara, 2015). Therefore, it is significant to determine whether malic acid was effective on growth and development of beef, dairy or combined-yield ruminant breeds and yield parameters of these ruminants (Toprak and Yılmaz, 2014). When the animal feeding studies about these natural preservatives were evaluated, it was observed that further in vivo studies were needed for them to be used as feed additives. The hypothesis of this study is that the use of malic acid, which is one of the organic acids used as a rumen modulator, in the rations of beef cattle (Limousine and Simmental) will have a significant effect on fattening performance, rumen bacteria and ciliate protozoa, and rumen fluid volatile fatty acids. This study was conducted to determine the effects of DL-malic acid supplementations into beef cattle rations of fattening performance, carcass traits and rumen parameters.

Material and Method

Throughout the experiments, animals were housed in a commercial farm (Ozsoy Fattening Farm in Bünyan town of Kayseri province) [with 10.05.2017 dated and 17/047 numbered decision of Animal Experiments Local Ethics Committee (HADYEK)].

A commercial dietary DL-malic acidproduced for ruminant nutrition was used in this study. This commercial supplement was included 65% of DL-malic acid, and 20% sodium-calcium salts. The product was powder form.

In present experiments, 47 male feeder cattle (19 Simmental (SIM) breed and 28 Limousine (LIM) breed) at the age of 14-16 months were used. Live weights of animal materials were determined at the beginning of the experiments and animal breeds were grouped as SIM control (n=9) and SIM DL-malic acid (n=10), LIM control (n=16) and LIM DL-malic acid (n=12). Animals were subjected to a 15-day adaptation period to adapt each other and their groups and then 120-day fattening program was applied to experimental groups.

The beef cattle were concentrated mix feed and high dry forage (alfalfa hay and wheat straw). The concentrated mix feed was the fattening finishing, which produced for beef cattle from a commercial company. The concentrated mix feed was in powder form and forages were chopped and mixed in feeding wagon.

In DL-malic acid group diets, different from the control group diets (SIM and LIM), DL-malic acid were spilled over the feeders in morning feeding as to have 30 g per animal. Number of beef cattle was taken into consideration in feeding and two meals were provided daily (at 08:00 and 18:00) as total mixed ration (TMR). Feed quantities were determined in accordance with NRC (1996) standards by taking dry matter, energy and the other nutrient requirements into consideration. Percentages of feedstuffs into total mixed ration and DM intake of beef cattle were given Table 1. Water was provided ad libitum to all animals.

Table 1. Percentages of feedstuffs into total mixed ration and DM intake of beef cattle

	Days of fattening study	
	0-70th days	**71-120th days**
	%, as DM in TMR	
Concentrate feed mixture	69.56	76.92
Alfalfa hay	8.65	5.76
Wheat straw	21.73	17.30
	DM intake, kg/day	
Wheat straw	2.00	2.00
Alfalfa hay	1.00	0.75
Concentrate feed mixture	9.00	10.00
Total TMR intake	12.00	12.75
	Nutrient matter intake	
CP	1309 g/day	1400 g/day
NDF	3270 g/day	3162 g/day
ME	31.2 Mcal/day	32.7 Mcal/day

DM: Dry Matter, CP: Crude Protein, NDF: Neutral Detergent Fiber

Chemical compositions of the feedstuffs used in study were analysed in accordance with the method specified in AOAC (2003). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) form structural fiber substances in feedstuffs were conducted in accordance with the method specified by Goering and Van Soest (1970) (Table 2).

Table 2. Chemical compositions of concentrated mix feed and forages used in study

Chemical compositions, %	**Feeds**		
	Concentrated mix feed	**Alfalfa hay**	**Wheat straw**
DM	91.16	93.35	92.85
CP	12.40	14.83	2.22
EE	3.27	1.08	0.94
CA	5.99	9.39	5.43
CC	5.75	31.09	42.79
ADF	6.07	36.51	49.81
NDF	15.14	44.48	72.11
ME, kcal/kg	2770	2104	2034

DM: Dry Matter; CP: Crude Protein; EE: Diethyl Ether Extract; CA: Crude Ash; CC: Crude Cellulose; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber; ADL: Acid Detergent Lignin; ME: Metabolic Energy.

Live weights of beef cattle were determined individually before morning feeding at the beginning, 30, 60, 90 and 120th (end) days of the experiments. Difference between weighing was expressed as live weight gain and dividing by the number of days was expressed as daily live weight gain. Following the slaughter and blood removal, head, skin, feet and entire internal organs were separated to get carcass weight. Carcass weight was then divided by slaughter weight to get hot carcass dressing percentage.

Body measurements [withers height (WH), chest girth (CG), rump height (RH) and body length (BL)] of beef cattle were taken at the beginning and end (120th day) of the experiments. Withers height and body length measurements were made with the use of measuring stick, rump height was measured with the use of a measuring compass and chest girth was measured with the use of a measuring tape. Withers height (WH) is the vertical distance from the ground to the highest point of withers; rump height (RH) is the distance between the outer sides of Tuber ichiies; body length (BL) is the horizontal distance from atric-ulus huneri to tuber ichii and chest girth (CG) is the circumference measured from back of forelegs to the level of forth rib (Brown et al., 1973).

For rumen fluid analyses, the rumen fluids were taken from 12 beef cattle (6 of each breed and 3 of each group) via stomach tube after 3 hours from feeding at the end of the experiments. The rumen fluid samples were preserved -20°C until the laboratory analyses.

The frozen samples were thawed at 4°C and centrifuged at 15000 g for 15 min using a micro-centrifuge (Gyrozen 1524, Daejeon, South Korea). After, the supernatants were transferred into the vials (Chromacol, Thermo Fisher Scientific, Orlando, FL, USA). Analysis of the VFAs was carried out using a

gas chromatograph (TRACE^{TM} 1300, Thermo Fisher Scientific, Orlando, FL, USA) equipped with an auto sampler (AI 1310, Thermo Scientific, Orlando, FL, USA), a polyethylene glycol column (length: 60 m, i.d: 0.25 mm, film thickness: 0.25 µm) (TG-WAXMS, Thermo Scientific, Orlando, FL, USA) and a flame ionization detector (FID). The carrier gas was helium at a constant flow rate of 1.5 ml/min. The injection volume was 0.5 µl. The samples were injected with split mode. The injection port temperature was 280° C. Oven temperature was programmed to increase from 160°C to 180°C at a rate 20°C/min. Air flow was 350 ml/min, and hydrogen flow was 35 ml/min. The temperature of FID detector was 300°C. Oven run time was 10 min. The concentrations of VFAs expressed as mmol/l were identified using a Xcalibur software programme (Thermo Scientific, Orlando, FL, USA). The concentrations of VFA [VA: valeric acid, IBA: *iso*-butyric acid, IVA: *iso*-valeric acid, PA: Propionic acid, BA: Butyric acid, AA: Acetic acid, TVFA: total volatile fatty acids] were given as mmol/L (Ersahince and Kara, 2017).

Total number of ciliated protozoan and generic composition were determined in accordance with the procedures specified in Dehority (1084). Fermentation liquid (0.1 ml) was fixed with 0.9 mL methyl green-formal-saline solution [100 ml formaldehyde (35 %), 900 ml distilled water, 0.6 g methyl green and 8.0 g NaCl]. Total number of ciliated protozoan and generic composition of diluted samples were determined with the use of large-bore pipette, Sedgewick Rafter lam and a microscope (Nikon Eclipse E-100, Holland). The following equation was used to calculate the number of protozoa in the rumen fluid: the numbers of cells in cm^3 (ml): 1000 x counted cells/counted total square x dilution x volume (Yıldız, 2001). Protozoa identification was made using figures determined by Ogimoto and Imai (1981). Protozoa species were counted up to 100 in the rumen fluid taken on the lam and the percentage of protozoa was calculated. Total number of bacteria was analysed with the use of a spectrophotometry (T80+UV/VIS Spectrophotometer, PG Instruments Ltd UK) (Kara et al., 2017).

Experimental data were subjected to variance analysis in accordance with repeated measures design. The difference between the control and experimental groups was analysed by one-way ANOVA test. Analyses were conducted in accordance with General Linear Model (GLM) procedure of SPSS (22.0) software by taking the Least Square Means (LSM) into consideration. Significance of number of bacteria, protozoa and volatile fatty acids were assessed through the use of Mann-Whitney U test. Since number of bacteria did not exhibit a normal distribution, relevant data were subjected to Log_{10} transformation. Summary data are presented as mean±standard error.

Results

Live weights (kg) and daily live weight gains (kg) of each breed for different periods are provided in Table 3. The differences in live weights of the groups were found to be significant on 30, 60 and 90th days of SIM breed (P<0.05), but insignificant in LIM breed (P>0.05). In SIM breed, live weights in on 30, 60 and 90thdays were respectively measured as 540.33 kg, 574.11 kg and 606.22 kg in control group and as 579.40 kg, 623.90 kg and 660.50 kg in DL-malic acid group (Table3). In SIM breed, live weights on 120th day of experimental groups were not found to be significant, but DL-malic acid group had a greater value than the control group (691.10 kg vs 635.44 kg) (Table3).

Table 3. Live weights and daily live weight gains of beef cattle fed with ration including malic acid

Live weight, kg	**Breeds**					
	Simmental			**Limousine**		
	Control (n=9)	**DL-Malic Acid (n=10)**	**P**	**Control (n=16)**	**DL-Malic Acid (n=12)**	**P**
Initial (0)	488.56±12.48	529.40±15.77	0.181	529.38±12.24	505.67±11.87	0.913
30th day	540.33±11.13	579.40±16.93	0.031	579.40±12.64	550.92±11.40	0.905
60th day	574.11±10.87	623.90±18.40	0.021	611.38±11.92	594.25±14.12	0.544
90th day	606.22±11.23	660.50±19.47	0.031	641.13±11.38	620.33±14.59	0.460
120th day	635.44±12.98	691.10±18.30	0.075	669.31±10.13	652.25±15.80	0.296
Daily live weight gain, kg						
0-30th days	1.724±0.124	1.667±0.133	0.436	1.644±0.109	1.508±0.098	0.373
30-60th days	0.992±0.103	1.309±0.144	0.302	0.961±0.059	1.273±0.110	0.112
60-90th days	0.893±0.078	1.017±0.089	0.314	0.827±0.090	0.725±0.085	0.177
90-120th days	0.712±0.150	0.745±0.072	**0.029**	0.687±0.082	0.779±0.077	0.917
0-120th days	1.041±0.073	1.147±0.051	0.313	0.992±0.046	1.039±0.049	0.911

The differences in daily live weight gains (kg) of the groups were found to be significant on 90-120th day of SIM breed (P<0.05), but insignificant in all periods of LIM breed (P>0.05) (Table3). In SIM breed, daily

live weight gains between 90-120th days was measured as 0.712 kg in control group and as 0.745 kg in DL-malic acid group. Animal body measurements (cm) at the beginning and end of the experiments are provided in Table 4. The differences in body measurements of the groups were not found to be significant in both breeds (P>0.05). The values for carcass traits of the groups are provided in Table 5. The differences in carcass weights of the groups at the end of fattening period were found to be significant in SIM breed (P<0.05) and the values in control and DL-malic acid groups were respectively measured as 374.27 kg and 417.20 kg. The differences in slaughter weight and carcass dressing percentage of the groups were not found to be significant in both breeds (P>0.05).

The count of bacteria in rumen fluids of the animals are provided in Table 8 and number of protozoa are provided in Table 6. The differences in number of bacteria and protozoa in rumen fluids of the groups were not found to be significant in both SIM and LIM breeds (P>0.05).

Table 4. Body measurements at the beginning and end of the experiments of beef cattle fed with ration including malic acid

Initial (1) and final (2) body measurements (cm)						
	Simmental			**Limousine**		
	Control (n=9)	**DL-Malic Acid (n=10)**	**P**	**Control (n=16)**	**DL-Malic Acid (n=10)**	**P**
WH-1	128.56±1.51	127.50±1.27	0.840	127.94±1.85	127.25±1.80	0.873
WH-2	137.33±0.79	138.50±1.04	0.607	137.88±1.30	1350.5±2.02	0.205
CG-1	185.44±1.86	189.10±2.46	0.205	194.62±2.36	187.25±1.93	0.710
CG-2	197.67±1.42	203.90±1.98	0.695	201.75±1.03	194.67±5.62	0.065
RH-1	61.89±1.34	57.10±1.61	0.710	63.31±1.07	55.00±1.57	0.397
RH-2	65.89±1.73	75.20±1.22	0.147	67.31±1.17	71.67±1.40	0.944
BL-1	106.89±1.73	112.80±2.20	0.132	108.44±1.13	111.08±1.66	0.166
BL-2	138.44±2.32	140.70±1.53	0.084	137.31±1.03	141.75±1.24	0.952

WH: Withers Height; CG: Chest Girth; RH: Rump Height; BL: Body Length.

Table 5. Carcass traits of beef cattle fed with ration including malic acid

Carcass performance	**Simmental**			**Limousine**		
	Control (n=9)	**DL-Malic Acid (n=10)**	**P**	**Control (n=16)**	**DL-Malic Acid (n=10)**	**P**
CW (kg)	374.27±8.91	417.20±13.13	**0.049**	398.29±7.19	390.10±10.41	0.449
SW (kg)	582.57±12.31	633.97±17.01	0.062	615.20±9.40	600.07±14.54	0.315
CDP (%)	64.22±0.20	65.74±0.32	0.083	64.71±0.30	64.97±0.21	0.271

CW: Carcass weight; SW: Slaughter Weight, DP: Carcass Dressing Percentage.

Table 6. Number of ciliated protozoa and total bacteria in rumen fluids of beef cattle fed with ration including malic acid

	Simmental			**Limousine**		
	DL-Malic Acid			**DL-Malic Acid**		
Number of protozoa	**0 g/day**	**30 g/day**	**P value**	**0 g/day**	**30 g/day**	**P value**
Total ruminal bacteria count	9.57±0.02	9.55±0.05	0.886	9.52±0.03	9.51±0.02	0.798
Percentages of individual ciliated protozoa in total ciliated protozoa in rumen fluid						
Entodinium (%)	0.44±0.01	0.40±0.00	0.057	0.42±0.01	0.45±0.03	0.505
Isotricha (%)	0.29±0.02	0.31±0.01	0.486	0.30±0.01	0.28±0.02	0.328
Dasytricha (%)	0.26±0.01	0.27±0.01	0.686	0.26±0.01	0.26±0.01	1.000
Diplodinium (%)	0.02±0.00	0.02±0.00	1.000	0.02±0.00	0.02±0.00	0.574
Number of ciliated protozoa in rumen fluid						
Entodinium (10^5/ml)	2.08±0.13	2.25±0.21	0.686	2.35±0.18	1.98±0.28	0.574
Isotricha (10^5/ml)	1.35±0.10	1.75±0.15	0.114	1.68±0.17	1.29±0.21	0.328
Dasytricha (10^4/ml)	1.25±0.10	1.50±0.17	0.486	1.43±0.10	1.19±0.21	0.195
Diplodinium (10^5/ml)	0.85±0.10	1.00±0.08	0.343	0.96±0.11	0.80±0.14	0.382
Total ciliated protozoa	4.76±0.23	5.60±0.53	0.343	5.55±0.44	4.53±0.70	0.328

It was determined that the addition of malic acid did not make any difference on the organic acid profile (valeric acid, iso-valeric acid, *iso*-butyric acid, propionic acid, butyric acid, acetic acid, total volatile fatty acids) in the rumen fluid of LIM and SIM beef cattle (P>0.05). On the other hand, when the effect of adding malic acid to the total of fattening cattle is compared; the addition of malic acid increased the molarities of acetic, butyric, propionic and total volatile fatty acids and AA/PA ratio in the rumen fluid (P<0.05). Molarities of valeric, *iso*-valeric and *iso*-butyric acids in the rumen fluid did not change with the addition of malic acid to beef cattle rations (P>0.05) (Tables 7 and 8). When comparing the percentage ratios of individual volatile fatty acids in TVFA in the rumen fluid, it was found that the percentage of acetic acid increased with the addition of malic acid; However, it was determined that the percentages of *iso*-butyric, *iso*-valeric and propionic acid in TVFA were decreased (P<0.05) (Table 8).

Discussion and Conclusion

In present study, effects of DL-malic acid supplementations (30 g/day per animal) into beef cattle diets on fattening performance, dressing percentage and rumen parameters were investigated. Previous researchers experimented different daily malic acid supplementation ratios (Alferez et al., 1978; Stullcup, 1979; Wang et al., 2009). The effects of DL-malic acid supplementation on live weights of LIM breed (combined yield breed) were found to be significant on 30, 60 and 90th days and effects on daily live weight gains were found to be significant on 90-120th days period. However, such effects were not found to be significant in LIM breed (meat yield breed) in SIM breed, average live weights on 30, 60 and 90th days were greater in DL-malic acid group than the control group. Live weight values on 30, 60 and 90th days were respectively measured as 540.33±11.13 kg, 574.11±10.87 kg and 606.22±11.23 kg in the control group and as 579.40±16.93 kg, 623.90±18.40 and

Table 7. Molarities of volatile fatty acids in rumen fluid of beef cattle

		Volatile fatty acids as mmol/L in rumen fluid						
MA	**Breed**	**VA**	**IBA**	**IVA**	**BA**	**PA**	**AA**	**TVFA**
0 g/day	**Simmental**	0.77±0.11	0.68±0.02	1.03±0.04	6.86±0.17	26.28±0.36	62.94±0.56	98.57±3.26
	Limousine	0.89±0.08	0.70±0.02	1.09±0.03	7.06±0.12	26.71±0.25	64.21±0.39	100.52±2.59
30 g/day	**Simmental**	0.88±0.11	0.68±0.02	1.04±0.03	8.13±0.14	29.51±0.27	76.28±0.51	116.53±3.26
	Limousine	0.90±0.09	0.67±0.01	1.01±0.05	7.53±0.11	27.57±0.29	73.62±0.42	111.32±2.59
Breed	**Simmental**	0.82±0.07	0.68±0.01	1.03±0.04	7.50±0.13	27.89±0.31	69.61±0.49	107.55±2.59
	Limousine	0.89±0.06	0.69±0.02	1.05±0.03	7.30±0.14	27.14±0.32	68.92±0.51	105.92±1.83
MA	**0**	0.85±0.07	0.69±0.02	1.08±0.03	6.99±0.15	26.57±0.31	63.79±0.48	99.87±2.43
	30	0.89±0.07	0.67±0.02	1.02±0.04	7.73±0.14	28.21±0.32	74.51±0.47	113.06±2.24
	SD	0.23	0.05	0.10	0.95	2.52	8.32	11.03
P values	**MA**	0.497	0.519	0.254	0.010	0.022	<0.001	<0.001
	Breed	0.395	0.685	0.598	0.537	0.383	0.764	0.610
	MA*Breed	0.559	0.422	0.156	0.215	0.171	0.401	0.267

MA: DL-Malic Acid, VA: Valeric Acid, IBA: Iso-butyric Acid, IVA: Iso-valeric Acid, PA: Propionic Acid, BA: Butyric Acid, AA: Acetic Acid, TVFA: Total Volatile Fatty Acids.

Table 8. Molarities of volatile fatty acids in rumen fluid of beef cattle

		Volatile fatty acids as % in TVFA						
MA	**Breed**	**VA**	**IBA**	**IVA**	**BA**	**PA**	**AA**	**AA/PA**
0 g/day	**Simmental**	0.78±0.12	0.69±0.02	1.05±0.03	6.96±0.16	26.70±0.31	63.80±0.48	2.39±0.04
	Limousine	0.90±0.08	0.71±0.01	1.11±0.04	6.99±0.14	26.61±0.28	63.86±0.45	2.41±0.05
30 g/day	**Simmental**	0.76±0.11	0.59±0.02	0.89±0.03	7.00±0.15	25.37±0.29	65.38±0.50	2.58±0.04
	Limousine	0.82±0.12	0.60±0.02	0.90±0.04	6.77±0.11	24.74±0.30	66.14±0.49	2.68±0.05
Breed	**Simmental**	0.77±0.10	0.64±0.01	0.97±0.03	6.98±0.14	26.03±0.30	64.59±0.50	2.48±0.06
	Limousine	0.86±0.11	0.65±0.02	1.00±0.04	6.88±0.14	25.68±0.32	65.00±0.55	2.54±0.05
MA	**0**	0.86±0.12	0.70±0.02	1.09±0.04	6.98±0.15	26.64±0.30	63.84±0.52	2.40±0.05
	30	0.80±0.12	0.60±0.02	0.90±0.04	6.85±0.14	24.95±0.31	65.89±0.53	2.65±0.06
	SD	0.26	0.08	0.16	0.57	1.48	2.13	0.22
P values	**MA**	0.567	<0.001	0.001	0.652	0.001	0.008	0.002
	Breed	0.340	0.554	0.481	0.641	0.427	0.549	0.399
	MA*Breed	0.743	0.925	0.601	0.549	0.541	0.608	0.563

MA: DL-Malic Acid, VA: Valeric Acid, IBA: İso-Butyric Acid, IVA: İso-Valeric Acid, PA: Propionic Acid, BA: Butyric Acid, AA: Acetic Acid, TVFA: Total Volatile Fatty Acids

660.50±19.47 kg in DL-malic acid group. In SIM breed, daily live weight gains on 30-60, 60-90, 90-120 and 0-120th days periods were greater in DL-malic acid group than in the control group, but the differences only in daily live weight gain of 90-120th day period were found to be significant. Relevant value was measured as 0.712±0.150 kg in the control group and as 0.745±0.072 kg in DL-malic acid group. Present findings for SIM breed comply with the findings of previous studies (Rossi and Vandoni, 2009). Contrary to present findings, Foley et al. (2009) working on feed consumption and performance traits of beef cattle indicated that malic acid supplementations into beef cattle diets negatively influenced feed consumptions and fattening performance. It was reported in similar previous studies conducted on calves (Seanson and Skullcap, 1984; Castillo et al., 2004) that malic acid supplementations into rations increased daily live weight gains and feed conversion ratios.It was reported in another study conducted on beef cattle (Martin et al., 1999) that malate supplementations into animal diets increased daily live weight gains and feed conversion ratios and improved fattening performance. Hackett et al. (1995) investigated the effects of post-partum 70 g per animal malic acid supplementations and reported that malic acid supplementations did not influence condition scores and live weights of the cows and birth weight and live weight gains of the calves. It was reported in previous studies conducted on lambs (Carro and Ranilla, 2003) that malic acid supplementation into high concentrate feed-containing lamb diets did not have significant effects on feed consumption, feed conversion and digestibility (CP, OM, ADF, NDF digestibility). Montaño et al. (1999) supplemented high concentration fattening finishing feeds with 80 g/day per animal and reported that malic acid supplementation balance rumen pH without any negative effects on digestion of ruminal nutrients (starch, fiber or protein) or microbial growth. Sniffen et al. (2006) indicated that malate supplementation into rumen through a cannula increased milk yield, but did not have significant effects on nutrient digestibility. Wang et al. (2009) supplemented Holstein dairy cows with 70, 140 and 210 g per animal malic acid and indicated that malic acid supplementations did not influence milk composition and nutrient consumption. It was also reported that malic acid supplemented groups had lower non-esterified fatty acids, beta-hydroxybutyrate and urea ketone contents and malic acid supplementations had positive effects on nutrient digestibility and energy use.

In present study, for SIM breed, DL-malic acid group had greater carcass weight, pre-slaughter live weight and dressing percentage (warm) values than the control group. However, differences only in carcass weights were found to be significant. On the other hand, for LIM breed, differences in these carcass traits were not found to be significant. It was reported in previous studies conducted on lambs (Süer and Kocabağlı, 2018) that malic acid supplementations did not have significant effects on animal performance and dressing percentages.

The differences in number of bacteria and protozoa in rumen fluids of the control and DL-malic acid groups were not found to be significant in both SIM and LIM breeds. Martin (1998) indicated that organic acids (malate and fumarate) increased lactic acid utilization capability of *S. ruminantium* as source of carbon and energy, in other words, removed lactic acid from the ambient through metabolization and thus prevented oxidase formation. Malic acid was indicated as the most efficient organic acid. It was reported that formic acid supplementation (0.7%) into rations reduced number of lactobacillus bacteria, coliform bacteria and yeasts (Canibe et al., 2001); potassium diformate salts (1.8%) and formic acid supplementations into the rations did not have significant effects on lactobacillus bacteria, but had significant effects on the other bacteria (Dibner and Buttin, 2002).

In the study, the molarity of TVFA in the rumen fluid of fattening cattle was at a level similar to the reference values (Kara, 2021). One of the volatile fatty acids, which are the end products of carbohydrate fermentation in the rumen, acetic acid rises with the increase of fermentation of structural carbohydrates (neutral detergent fiber, acid detergent fiber and other dietary fiber components) (NRC, 1996). In the presented study, the increase in acetic acid molarity with the addition of malic acid to the ration shows that malic acid has a positive effect on rumen fermentation. In addition, propionic acid and butyric acid increase in correlation with the increase in the fermentation level of starch and other digested nutrients in the rumen (NRC, 1996). The increase in the propionic and butyric acid molarity of malic acid with dietary malic acid shows that malic acid has a positive effect on the ruminal fermentation of the feed. In the study, the ratios of *iso*-valeric and iso butyric acid in rumen fluid decreased with the addition of malic acid to the ration. Kara (2015) stated that *iso*-valeric acid concentration in the organic acids of the silage was decreased with maleic acid addition to silage. Cong et al. (2009) stated that malic acid supplementation (70 g, 140 g and 210 g) to SIM rations beef ration increased TVFA concentration and decreasing ruminal pH, acetate, propionate in rumen fluid. It was reported by the same researchers in an in vitro study on ruminants that 100 g malic acid supplementation into the ration and additional 50 g malic acid supplementation at the middle of the day did not influence VFAs, but 50 g malic acid increased microbial N production and efficiency, thus indicated that daily supplementation of 50 g malic acid positively influenced rumen fermentation and microbial population. In another study, Nisbet and Martin (1990) indicated that malic

acid activated in vitro DL-lactic acid utilization and increased pH, total VFA and propionate percentage in rumen fluid. Mohammed et al. (2004) reported that malate supplementation into the ruminant diets increased ratios of propionic acid and total VFA. It was indicated in a previous study conducted on lambs (Kung et al., 1982) that malic acid supplementations influenced acetic and butyric acid quantities produced in rumen. In the study, the positive effect of malic acid on rumen fermentation is similar to previous studies.

Consequently, DL-malic acid supplementation (30 g/ day) to beef cattle ration varied with the cattle breeds in terms of fattening performance, while such supplementations had positive effect on fattening in Simmental breed. Besides, the increase in molarities of TVFA and acetic, propionic and butyric acids, which are indicators of fiber and carbohydrate fermentation efficiency in the rumen, shows that malic acid has a positive effect on feed digestion in the rumen.

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