https://doi.org/10.17221/100/2017-VETMED

Estimated ruminal digestion values and digestion end-products of concentrated mix feed after *in vitro* treatment with propionic acid

K. Kara*

Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

*Corresponding author: karakanber@hotmail.com

ABSTRACT: This study was aimed at determining the effects of propionic acid supplementation at doses of 0 (control group, PA0), 12, 24, 48 and 96 mM (PA12, PA24, PA48, and PA96) to concentrated mix feed on *in vitro* cumulative total gas production, methane emission, gas kinetics (potential gas production, $(a + b)_{gas}$ and gas production rate, c_{gas}), estimated digestibility, estimated energy value and the end-products and variables of *in vitro* digestion (total bacteria count, the number of ciliate protozoa, volatile fatty acids, pH value and ammonia-N). Digestion treatments were carried out in an anaerobic *in vitro* fermenter for up to 96 h. The *in vitro* cumulative total gas production, $(a + b)_{gas}$, estimated metabolic energy, estimated net energy lactation and estimated organic matter digestibility and ammonia-N concentration were decreased by propionic acid up to 96 mM ($P < 0.05$). In the *in vitro* fermenter fluid, total bacteria count, the total numbers of ciliate protozoa and the individual numbers of some ciliate protozoa (*Entodiniinae*, *Isotricha spp*. and *Diplodiniinae*) ($P < 0.01$) decreased linearly with increasing concentrations of dietary propionic acid. The total molar concentrations of volatile fatty acids decreased in response to propionic acid supplementation ($P < 0.001$). Dietary propionic acid elicited linear increases in the molar concentrations of propionic acid ($P < 0.001$) and butyric acid ($P < 0.01$) as proportions of total volatile fatty acids of the *in vitro* fermenter fluid. In contrast, molar proportions of acetic acid, the c_{gas}, pH values and the numbers of *Dasytricha* sp. were not affected by dietary propionic acid supplementation ($P > 0.05$). The addition of 12–96 mM propionic acid to concentrated mix feed decreased methane emission from the rumen and negatively affected microbiota count, feed digestibility, proteolysis, and molar volatile fatty acid values in the rumen environment.

Keywords: alternative dietary supplement; digestibility; fermentation; *in vitro* gas production

Organic acids are used as an alternative to antibiotic performance stimulants in animal diets. They exert positive effects on feed quality and performance by changing microbiota balance/counts and by altering physiological processes in the digestive canal (Martin 1998). Generally, organic acids with specific antimicrobial activity are short-chain acids (C1–C7) and have a pKa of between three and five (Papatsiros et al. 2013). Some dicarboxylic acids (fumarate, malate and maleic acid) are propionate precursors in the pathway from succinate to propionate and act as H_2 acceptors (Callaway and Martin 1996; Castillo-Gonzalez et al. 2014). These propionate precursors act as alternative electron sinks, and they may compete with methane generation in the rumen (Callaway and Martin 1996; Kara 2015). In previous studies, it was reported that propionate precursors can act as rumen modulators due to anti-methanogenic effects, their role in reducing total volatile fatty acid (VFA) levels and based on their modulation of the molar proportion of propionic acid (PA) (Pandey et al. 2012; Kara 2015; Kara et al. 2015a). At the same time, some of these dietary acids can exert adverse effects on rumen fermentation by inhibiting fibre substance digestion or decreasing the number of ciliate protozoa (Sirohi et al. 2012). The effects of propionate precursors (fumarate and malate) on methane production in the rumen may depend on the diet type (forage or concentrate) and the level of organic acid (Lopez et al. 1999a; Lopez et al. 1999b; Newbold et al. 2002; Garcia-Martinez et al.

https://doi.org/10.17221/100/2017-VETMED

2005). Pandey et al. (2012) reported that PA addition (10 mM) to the incubation medium did not change dry matter digestibility, fibre digestibility and methane production, but increased molar VFA concentrations and propionate concentrations *in vitro*. Although there are studies on the use propionate precursors in ruminant diets, studies on the effects of PA on ruminal fermentation parameters are scarce. Limited levels of organic acids have been found to have a positive effect on feed digestion in ruminants. However, it has been determined that high levels of organic acids have a negative effect on some ruminal fermentation values.

I hypothesised that the addition of high levels of propionic acid to concentrated feed mixture would have a negative effect on digestibility values. The current study was aimed at determining the effects of supplementation of up to 96 mM PA to concentrated mix feed on *in vitro* cumulative total gas production, methane emission, gas kinetics (potential gas production; a (gas production from the immediately soluble fraction, ml) + b (gas production from the insoluble fraction, ml))$_{gas}$ and gas production rate; c_{gas}), estimated digestibility, estimated energy value and the end-products and variables of *in vitro* digestion (total bacteria count, the number of ciliate protozoa, VFAs, pH value and ammoniacal-N).

MATERIAL AND METHODS

Propionic acid. A commercially produced PA feed additive (Luprosil liquid, BASF) was used. This feed additive is infinitely miscible with water and is a clear liquid with pungent odour, which contains a minimum of 99.5% propionic acid (product code: 10002210, molecular formula CH_3CH_2COOH, molar mass 74.01 g/mol). It has a pH of 2.5 (at 100 g/l H_2O). The volumes of commercial PA feed supplement of 1, 2, 4 and 8 ml contained 0.869 g, 1.74 g, 3.47 g and 6.95 g propionic acid, respectively. These volumes of supplement had propionic acid concentrations of 12, 24, 48 and 96 mM, respectively.

Substrate ingredients. The beef cattle concentrated mix feed consisted of 53.3% barley grain, 20% corn grain, 8% wheat brain, 10% cotton seed meal, 5% sugar beet molasses, 2.5% limestone, 0.5% di-calcium phosphate and 0.7% sodium chloride on a DM basis.

Wet chemical analysis of mix feed. The feed was ground down and passed through a 1-mm sieve (IKA MF10.1, Germany) for analysis. The dry matter (DM), ash, crude protein (CP), ether extract (EE) and crude fibre (CF) contents of the diet were analysed using AOAC methods (AOAC 1980 and 1990; methods 7.066–7.070; method 954.01; method 14.081; method 942.05; method 920.39). Fibrous plant cell wall contents (neutral detergent fibre; NDF, acid detergent fibre; ADF and acid detergent lignin; ADL) were analysed using a fibre analyser (Van Soest et al. 1991). Non-fibrous carbohydrate contents (NFC) were calculated using NDF, CP, EE and ash values (NRC 2001). The chemical composition and ingredients of the concentrated mix feed for beef cattle is presented in Table 1 (Kara et al. 2018).

***In vitro* gas production technique**. The *in vitro* digestion technique was performed using four different doses (12, 24, 48 and 96 mM) of PA supplementation (PA12, PA24, PA48 and PA96 groups, respectively) to feed. PA was not added to the control group (PA0 group).

For *in vitro* digestion, I used fresh rumen fluid which was obtained from three beef cattle fed a diet containing forage feed (about 20% of diet on a DM basis;) and concentrate feed (about 80% of diet on a DM basis). The forages consisted of corn silage + meadow hay + wheat straw + alfalfa hay. Rumen fluid was placed into a thermos under constant CO_2 gas and then filtered using muslin with a 1–5 µm pore diameter to obtain an inoculum. In the *in vitro* gas production technique (Menke et al. 1979), concentrated mix feed samples (with or without PA) (0.200 ± 0.010 g) (substrate) were incubated with rumen fluid inoculum (10 ml) and buffer mixture (20 ml) in an aerobic glass fermenter (with 100 ml volume, Model Fortuna, Germany) at 39 °C for up to 96 h, in triplicate. Three blank

Table 1. Analysis of the chemical content of the concentrated mix feed

Analysed parameter	Dry matter basis (%)
Crude protein	12.70
Non-fibrous carbohydrate contents	59.57
Neutral detergent fibre	17.90
Acid detergent fibre	7.90
Acid detergent lignin	5.60
Crude fibre	5.80
Ether extract	3.13
Ash	6.70

https://doi.org/10.17221/100/2017-VETMED

glass fermenters (no samples) were incubated as correction values.

Determination of gas production values. The total gas volume and the produced substrates were read from the volume lines on the glass fermenter at 3, 6, 12, 24, 48, 72 and 96 h. The amount of methane gas as a proportion of total gas produced at 24 h was determined in an infrared methane measurement device (Sensor, Europe GmbH, Erkrath, Germany) according to Kara et al. (2015b). Cumulative gas production data were fitted to the exponential equation of Orskov and McDonald (1979):

$$y = a + b\ (1 - \exp^{-ct})$$

where: a = gas production from the immediately soluble fraction (ml); $a + b$ = potential gas production (ml); b = gas production from the insoluble fraction (ml); c = gas production rate constant; t = incubation time (h); y = gas produced at time t

The *in vitro* gas production kinetics for each group were calculated using computer software (Fig P, Biosoft, Cambridge, United Kingdom).

Estimated energy and digestion levels. The estimated metabolisable energy (ME), net energy lactation (NE_L) and estimated organic matter digestibility (OMD) values for mix feeds were calculated using the gas production values and CP, EE and ash contents (Menke and Steingass 1987).

The characteristics of *in vitro* fluid in fermenters. I determined the basic chemical values of the fluid in the *in vitro* glass fermenters which measured methane production at 24 h of incubation. The pH value of the filtered *in vitro* fermentation fluid was determined using a digital pH meter (Mettler Toledo S220, Switzerland). To determine the ammoniacal-N (NH_3–N, mg/dl) concentration, fermentation fluid was centrifuged at 1600 × *g* for 5 min and then distilled in a distillation system. The distillate in 4% (w : w) of H_3BO_3 was titrated with 0.1 N of HCl (Makkar and Becker 1996).

The 10 ml of fermentation fluid in each fermenter were transferred to tubes. The generic compositions and total numbers of ciliate protozoa in fermentation fluid samples were determined according to Dehority (1978). Ruminal total bacteria count was determined using a spectrophotometer. The 100 µl of ruminal fermentation fluid were diluted with 35% formaldehyde (900 µl). The absorbance of the mixture was read at 660 nm wavelength using an UV/VIS spectrophotometer (SI Analytics – Xylem Analytics Germany Sales GmbH & Co. KG, Mainz, Germany) (Minato and Suto 1981).

The VFA concentration of *in vitro* ruminal fermentation fluid (acetic, propionic and butyric acids; mmol/l in fluid and % of the total VFAs) was measured using a gas chromatograph (TRACE™ 1300, Thermo Fisher Scientific, Orlando, USA) device equipped with a flame ionisation detector (TG-WAXMS, Thermo Scientific, Orlando, USA) (Ersahince and Kara 2017).

Statistical analysis. One-way analysis of variance was performed for homogeneous variances using GLM procedures to test treatment differences. Data were analysed according to the model:

$$Y_{ij} = \mu_{ij} + S_i + e_i$$

where: e_i = standard error term; S_i = i^{th} effect of 12, 24, 48 and 96 mM propionic acid supplementation to feed on the studied variables; Y_{ij} = general mean common for each parameter under investigation; μ_{ij} = mean of propionic acid supplementation for each studied variable

Table 2. Impact of propionic acid addition to the feed on *in vitro* cumulative gas production

Gas production times (h)	Propionic acid doses					SD	Statistical significance	
	0 mM	12 mM	24 mM	48 mM	96 mM		linear	quadratic
3	19.33	12.00	11.16	10.16	15.50	5.12	*	***
6	42.00	26.83	26.00	24.16	22.83	7.63	***	***
12	55.33	36.83	36.00	33.16	31.83	10.21	***	***
24	69.33	51.75	47.41	42.08	44.75	10.52	***	***
48	76.00	67.91	51.91	45.91	46.25	12.75	***	***
72	78.66	71.91	53.25	46.91	52.37	13.06	***	***
96	81.00	73.25	52.58	47.25	53.70	13.89	***	***

linear = linear effect of propionic acid dose, quadratic = quadratic effect of propionic acid dose
*P < 0.05, ***P < 0.001

https://doi.org/10.17221/100/2017-VETMED

Table 3. Ruminal fermentation parameters and gas kinetics of feed in response to supplementation with different doses of propionic acid

Items	Propionic acid doses					SD	Statistical significance	
	0 mM	12 mM	24 mM	48 mM	96 mM		linear	quadratic
c_{gas}	0.13	0.05	0.10	0.12	0.07	0.03	ns	ns
$(a + b)_{gas}$	75.80	74.94	53.41	50.52	45.16	13.75	***	ns
Methane (%)	24.63	20.80	20.46	19.75	19.66	2.05	***	***
ME (MJ/kg DM)	12.19	9.30	8.62	7.65	8.27	1.72	***	***
NE_L (MJ/kg DM)	7.77	5.66	5.16	4.45	4.91	1.26	***	***
OMD (% DM)	79.87	61.62	57.29	51.12	55.12	10.92	***	***
NH_3–N (mg/dl)	114.49	51.45	46.55	48.84	45.24	27.46	***	***
pH	6.77	6.78	6.78	6.80	6.78	0.02	ns	ns

$(a + b)_{gas}$ = *in vitro* potential gas production (ml for 0.2 g as DM), c_{gas} = *in vitro* gas production rate (0.2 g as DM), DM = dry matter, linear = linear effect of propionic acid dose, ME = metabolisable energy, NE_L = net energy lactation, OMD = estimated organic matter digestibility, quadratic = quadratic effect of propionic acid dose

***$P < 0.001$

The means were separated by Tukey's multiple range test at $P < 0.05$.

Linear relations among the studied indicators were determined by calculating Pearson correlation coefficients in SPSS 17.0 software (IBM Corp., Armonk, USA).

RESULTS

The values of cumulative gas production at 96 h of incubation were 81 ml/0.2 g dry matter for 0 mM PA addition and 73.25–47.25 ml/0.2 g dry matter for 12–96 mM PA addition (Table 2). The $(a + b)_{gas}$, c_{gas}, cumulative gas production, methane emission, estimated energy values (ME, NE_L), estimated OMD levels and ammoniacal-N concentrations were decreased by PA addition to feed (Tables 2 and 3). Ruminal pH and gas production rates did not change in PA groups compared to the control group (Table 3).

Total bacteria counts were decreased in both linear and quadratic fashions by the addition of PA. Total ciliate protozoa, *Entodiniinae*, *Isotricha* spp. and *Diplodiniinae* protozoa numbers decreased linearly with increasing dietary PA. The numbers of *Dasytricha* sp. protozoa were not affected by dietary PA supplementation (Table 4).

The total molar concentration of VFAs decreased with 1–8 ml/kg dietary PA supplementation, in both linear and quadratic fashions. Dietary PA resulted in linear increases in the molar concentra-

Table 4. Numbers of ruminal bacteria (× 10^8/ml) and protozoa (× 10^4/ml) in feed in response to different doses of propionic acid

Items	Propionic acid doses					SD	Statistical significance	
	0 mM	12 mM	24 mM	48 mM	96 mM		linear	quadratic
Total bacteria	1.28	0.86	0.83	0.89	0.85	0.20	**	**
Total ciliate protozoa	68.80	50.92	37.28	34.66	29.86	16.03	***	ns
Isotricha spp.	0.36	0.25	0.22	0.16	0.15	0.10	**	ns
Dasytricha sp.	0.47	0.48	0.46	0.48	0.43	0.04	ns	ns
Diplodiniinae	23.46	14.33	11.23	11.73	8.00	6.67	**	ns
Entodiniinae	44.46	35.83	25.36	22.33	21.26	9.82	***	ns

linear = linear effect of propionic acid dose, quadratic = quadratic effect of propionic acid dose

**$P < 0.01$, ***$P < 0.001$

https://doi.org/10.17221/100/2017-VETMED

Table 5. Ruminal total volatile fatty acids (VFA; mmol/l) and molar proportions of individual volatile fatty acids (% of total VFAs) in response to the addition of different doses of propionic acid to feed

Items	Propionic acid doses					SD	Statistical significance	
	0 mM	12 mM	24 mM	48 mM	96 mM		linear	quadratic
Total VFA's	79.23	48.50	49.07	46.24	44.53	13.50	***	***
Acetic acid (A)	45.88	45.84	44.97	44.23	44.86	1.40	NS	NS
Propionic acid (P)	22.98	25.24	26.63	27.26	27.38	2.05	***	NS
Butyric acid (B)	22.96	20.81	19.74	20.12	19.76	1.65	**	NS
OVA	8.18	8.11	8.66	8.12	8.00	0.61	NS	NS
(A + B)/P	3.00	2.64	2.43	2.36	2.36	0.28	***	*

A = molar proportion of acetic acid; B = molar proportion of butyric acid; linear = linear effect of propionic acid dose; OVA = valeric acid, iso-valeric acid, iso-butyric acid; P = molar proportion of propionic acid; quadratic = quadratic effect of propionic acid dose;
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

tions of PA and butyric acid as proportions of total VFAs. In contrast, molar proportions of acetic acid and other volatile acids (iso-butyric acid, valeric acid and iso-valeric acid) in total VFAs did not change with dietary PA supplementation. Dietary PA reduced the (A + B)/P ratio in both linear and quadratic fashions (see Table 5).

Calculation of Pearson correlation coefficients between PA and *in vitro* digestibility values (Table 6) revealed that methane production was positively correlated with the OMD, NH_3–N, microbial count and VFA concentration but was negatively correlated with the molar proportion of PA. The OMD was positively correlated with the NH_3–N, microbial count and VFA concentration but was negatively correlated with the molar proportion of PA. The NH_3–N was positively correlated with the total bacteria count, total protozoa number, $(a + b)_{gas}$ and VFA concentration but was negatively correlated with the molar proportion of PA. Total protozoa were positively correlated with the $(a + b)_{gas}$ but negatively correlated with the molar proportion of PA. The potential gas production was positively correlated with the total VFA

Table 6. Correlation coefficients (*r*) of the relationships among *in vitro* digestion values in response to addition of different doses of propionic acid to feed

	OMD	NH_3–N	TBact	TProt	$(a + b)_{gas}$	mVFA	A	P	B	(A + B)/P
MP	0.795**	0.857**	0.743**	0.799**	0.608	0.869**	0.282	–0.640*	0.559*	0.690**
OMD	1	0.884**	0.775**	0.759**	0.603	0.951**	0.122	–0.704**	0.721**	0.743**
NH_3–N		1	0.851**	0.784**	0.660*	0.970**	0.252	–0.760**	0.762**	0.817**
TBact			1	0.704**	0.532	0.864**	0.170	–0.593*	0.566*	0.643**
TProt				1	0.897**	0.807**	0.300	–0.701**	0.648**	0.733**
$(a + b)_{gas}$					1	0.664*	0.743*	–0.865**	0.624	0.880**
VFA						1	0.273	–0.778**	0.728**	0.828**
A							1	–0.432	–0.071	0.489
P								1	–0.828**	–0.990**
B									1	0.810**

*Correlation coefficient is significant at the 0.05 level, **correlation coefficient is significant at the 0.01 level
$(a + b)_{gas}$ = *in vitro* potential gas production (ml for 0.2 g as dry matter), A = molar proportion of acetic acid, B = molar proportion of butyric acid, MP = methane production at 24 h, mVFA = molarity of total volatile fatty acids, OMD = estimated organic matter digestibility, P = molar proportion of propionic acid, TBac = total bacteria count, TProt = total protozoa number, VFA = volatile fatty acid

https://doi.org/10.17221/100/2017-VETMED

concentration, molar proportion of acetic acid and (A + B) : P ratio but was negatively correlated with the molar proportion of PA (Table 6).

DISCUSSION

Levels of *in vitro* total gas production are determined by factors such as digestion level of the substrate, dietary supplements (organic acids, condensed tannin and other secondary compounds), the presence of easily soluble/fermentable carbohydrates, the bacterial and protozoan populations of the donor rumen fluid and the quality (constancy of anaerobicity, temperature, etc.) of the fermentation environment (Kara et al. 2015a; Kara et al. 2015b). In the current study, the decrease in cumulative total gas production and estimated digestibility parameters (gas kinetics, OMD, ME and NE_L) may be connected with antimicrobial effects on ruminal total bacteria and total protozoa number and specifically on *Isotricha* spp., *Entodiniinae* and *Diplodiniinae*. Further, the decrease may have been due to the effect of PA in reducing the molar concentrations of VFAs in the rumen fluid.

Organic acids, such as maleic (*cis*-butenedioic) acid, fumaric acid, malic acid, lactic acid and pyruvic acid involved in the succinic acid to propionic acid pathway, play important roles as precursors to propionate. If the levels of these acids or of propionic acid in the rumen environment are increased by dietary organic acid supplementation or normal ruminal fermentation, ruminal propionate concentrations will be high and ruminal methane emission will then be reduced (Kara 2015). Previous researchers have stated that the archaeal population of the rumen is responsible for ruminal methane production (Hook et al. 2010). In the present experiment, PA addition resulted in a linear decrease in ruminal methane production by up to about 20%. The decrease in methane production was positively correlated with total microorganism count, OMD, NH_3–N, total VFAs and the molar proportion of butyric acid in rumen fluid. Kara (2015) reported that *in vitro* gas production, ME and OMD values of corn silage did not change in response to supplementation with 0.5–1.5% maleic acid as a ruminal precursor of propionate in the ensiling stage.

The genera and number of total protozoa found in the rumen environment change as associated with nutrient composition and digestibility capacity of feed (Veira 1986). In the current study, the total number of ciliate protozoa was significantly decreased. Donmez et al. (2003) reported that formic acid addition to silage resulted in a decreased number of total protozoa in rumen fluid. The mechanism underlying the decrease in protozoa and bacteria may involve changes in cell membrane permeability in response to organic acids and resulting cell lysis (Francis et al. 2002). The decreases in total protozoa number (*Isotricha* spp., *Diplodiniinae* and *Entodiniinae*) and total bacteria count elicited by PA supplementation was similar to those reported by Pandey et al. (2012). In the present study, total protozoa number was reduced by up to about 57% with 1–8 ml/kg PA supplementation to concentrated mix feed. Pandey et al. (2012) determined that 10 mM PA supplementation to highly concentrated feed (20% roughage + 80% concentrate) decreased total protozoa numbers by up to 75%.

Dicarboxylic acids such as fumaric, malic and tartaric acids, which are propionate precursors in the pathway from succinate to propionate, act as H_2 acceptors, which in turn results in a decrease methane production (Callaway and Martin 1996; Sirohi et al. 2012). Previous studies using fumarate, malate and maleic acids as ruminal precursors of propionate reported decreased ruminal methane production (Martin 1998; Lopez et al. 1999a; Lopez et al. 1999b; Tejido et al. 2005; Kara 2015). In another study, it was determined that for a high-fibre diet (80% roughage + 20% concentrate) supplemented with PA (10 mM) methane production was reduced by up to 12.4% (Pandey et al. 2012). In contrast to these results, the reduction (by up to about 20%) in methane production in the current study may be connected with PA's antimicrobial effects on rumen bacteria and protozoa rather than with its role as a H_2 acceptor. A decrease in the numbers of rumen microorganisms results in decreased OMD and in turn lower levels of gas production and of VFAs (Sirohi et al. 2012). Methane production was positively correlated with the OMD, NH_3–N, numbers of microorganisms in the rumen, total VFA concentration and the (A + B) : P ratio. In agreement with our fermentation results, previous studies have reported *Holotrich* protozoa to be important for methanogenesis in the rumen (Belanche et al. 2012; Belanche et al. 2015; Kara et al. 2018).

A low digestibility of feed in the rumen results in decreased molar VFA concentrations in rumen

https://doi.org/10.17221/100/2017-VETMED

fluid. In the current experiments, the concentration of total VFAs and the molar proportion of butyric acid was decreased, while the molar proportion of PA was increased bin response to PA addition. These findings are similar to those of Li et al. (2015) who used 2% DL-malate or 2% fumarate as ruminal propionate precursors. The decreased total molar levels of VFAs and individual VFAs in rumen fluid may be caused by antimicrobial effects on ruminal bacteria and the ciliate protozoa community. In a previous study, it was shown that the concentration of total ruminal VFAs increased linearly with increasing numbers and types of ciliate protozoa in rumen fluid (Belanche et al. 2015).

In the rumen environment, a certain proportion of true protein (ruminal degradable protein) and mostly non-protein nitrogenous (urea) compounds in feed are degraded by the rumen microbiota (proteolysis) and are converted to ammoniacal-N. However, a certain portion of the protein compounds are not degraded in the rumen and, thus, do not increase ruminal ammoniacal-N concentrations. The positive effects on animal performance of some dietary organic acids are explained in part by decreased levels of ruminal degradable protein, which can result in an increased flow of ruminal undegradable protein to the abomasum and duodenum. This increased flow of ruminal undegradable protein inhibits the release of and lowers the concentration of ammoniacal-N in the rumen environment (Dibner and Buttin 2002; Baytok et al. 2005; Jaakkola 2006). In the present study, with increasing levels of PA supplementation (up to 96 mM) a linear decrease (by as much as 60%) in ruminal ammoniacal-N concentrations were observed. Decreased concentrations of ammoniacal-N in rumen fluid may be related to the negative effects of PA on bacterial counts and on proteolytic *Entodiniinae* and *Diplodiniinae* protozoa which generate ammoniacal-N (Ivan et al. 2000; Castillo-Gonzalez et al. 2014). In contrast, Li et al. (2015) reported that concentrations of ammoniacal-N in the rumen fluid were not affected by supplementation with 2% DL-malate or 2% fumarate as ruminal precursors of propionate.

In conclusion, the results reported here demonstrated that addition of propionic acid to concentrated beef mix feed decreased the numbers of microbes in the rumen, organic matter digestibility, levels of degradable protein compounds, molar concentrations of VFAs and ruminal methane emissions. The effects of 12–96 mM propionic acid supplementation to concentrated feed should be validated in more detailed *in vivo* studies in the future. The results of future *in vivo* studies and of current *in vitro* studies will paint a clearer picture of the effects of PA addition to feed.

Acknowledgement

I thank Mr. Cem Soyturk (Sinerji Tarim Ltd. Sti/ Izmir, Turkey) for providing propionic acid feed additives for Luprosil liquid (BASF The Chemical Company).

REFERENCES

AOAC – Association of Official Analytical Chemists (1980): Official Methods of Analysis. 13th edn. Association of Official Analytical Chemists, Inc., Arlington.

AOAC – Association of Official Analytical Chemists (1990): Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists, Inc., Arlington.

Baytok E, Aksu T, Karsli MA, Muruz H (2005): The effect of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. Turkish Journal of Veterinary and Animal Sciences 29, 469–474.

Belanche A, Fuente G, Moorby JM, Newbold CJ (2012): Bacterial protein degradation by different rumen protozoal groups. Journal of Animal Science 90, 4495–4504.

Belanche A, Fuente G, Newbold CJ (2015): Effect of progressive inoculation of fauna-free sheep with holotrich protozoa and total-fauna on rumen fermentation, microbial diversity and methane emissions. FEMS Microbiology Ecology 91, doi: 10.1093/femsec/fiu026.

Callaway TR, Martin SA (1996): Effects of organic acid and monensin treatment on in vitro mixed ruminal microorganism fermentation of cracked corn. Journal of Animal Science 74, 1982–1989.

Castillo-Gonzalez AR, Burrola-Barraza ME, Dominguez-Viveros J, Chavez-Martinez A (2014): Rumen microorganisms and fermentation. Archivos de Medicina Veterinaria 46, 349–361.

Dehority BS (1978): Specificity of rumen ciliate protozoa in cattle and sheep. The Journal of Eukaryotic Microbiology 25, 509–513.

Dibner J, Buttin P (2002): Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. The Journal of Applied Poultry Research 11, 453–463.

https://doi.org/10.17221/100/2017-VETMED

Donmez N, Karsli MA, Cinar A, Aksu T, Baytok E (2003): The effects of different silage additives on rumen protozoan number and volatile fatty acid concentration in sheep fed corn silage. Small Ruminant Research 48, 227–231.

Ersahince AC, Kara K (2017): Nutrient composition and in vitro digestion parameters of Jerusalem artichoke (Helianthus tuberosus L.) herbage at different maturity stages in horse and ruminant. Journal of Animal and Feed Sciences 26, 213–225.

Francis G, Kerem Z, Makkar HPS, Becker H (2002): The biological action of saponins in animal systems: a review. British Journal of Nutrition 88, 587–605.

Garcia-Martinez R, Ranilla MJ, Tejido ML, Carro MD (2005): Effects of disodium fumarate on in vitro rumen microbial growth, methane production and fermentation of diets differing in their forage:conc entrate ratio. British Journal of Nutrition 94, 71–77.

Hook SE, Wright AD, McBride BW (2010): Methanogens: methane producers of the rumen and mitigation strategies. Archaea 2010, doi: 10.1155/2010/945785.

Ivan M, Neill L, Forster R, Alimon R, Rode LM, Entz T (2000): Effects of Isotricha, Dasytricha, Entodinium, and total fauna on ruminal fermentation and duodenal flow in wethers fed different diets. Journal of Dairy Science 83, 776–787.

Jaakkola S (2006): Effects of restriction of silage fermentation with formic acid on milk production. Agricultural and Food Science 15, 200–218.

Kara K (2015): In vitro methane production and quality of corn silage treated with maleic acid. Italian Journal of Animal Science 14, 714–722.

Kara K, Aktug E, Cagri A, Guclu BK, Baytok E (2015a): Effect of formic acid on in vitro ruminal fermentation and methane emission. Turkish Journal of Agriculture – Food Science and Technology 3, 856–860.

Kara K, Guclu BK, Baytok E (2015b): Comparison of nutrient composition and anti-methanogenic properties of different Rosaceae species. Journal of Animal and Feed Sciences 24, 308–314.

Kara K, Ozkaya S, Erbas S, Baytok E (2018): Effect of dietary formic acid on the in vitro ruminal fermentation parameters of barley-based concentrated mix feed of beef cattle. Journal of Applied Animal Research 46, 178–183.

Li XZ, Park BK, Shin JS, Choi SH, Smith SB, Yan CG (2015): Effects of dietary linseed oil and propionate precursors on ruminal microbial community, composition, and diversity in Yanbian Yellow Cattle. PloS One 10, doi: 10.1371/journal.pone.0126473.

Lopez S, Newbold CJ, Bochi-Brum O, Moss AR, Wallace RJ (1999a): Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal in vitro. South African Journal of Animal Science 29, 106–107.

Lopez S, Valdes C, Newbold CJ, Wallace RJ (1999b): Influence of sodium fumarate addition on rumen fermentation in vitro. British Journal of Nutrition 81, 59–64.

Makkar HPS, Becker K (1996): Nutritional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. Animal and Feed Science Technology 63, 211–228.

Martin SA (1998): Manipulation of ruminal fermentation with organic acids: A review. Journal of Animal Science 76, 3123–3132.

Menke KH, Steingass H (1987): Food energy content estimates from in vitro gas production in rumen fluid and from chemical analysis. II. Regression equations (in German). Uebersicht zur Tierernaehrung 15, 59–94.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979): The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. Journal of Agricultural Science 93, 217–222.

Minato H, Suto T (1981): Technique for fractionation of bacteria in rumen microbial ecosystem. IV. Attachment of rumen bacteria to cellulose powder and elution of bacteria attached to it. The Journal of General and Applied Microbiology 27, 21–31.

NRC – Nutrient Requirements of Dairy Cattle (2001): Nutrient requirements of dairy cattle. 7th edn. Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture and Natural Resources, National Research Council. National Academy Press, Washington, D.C.

Newbold CJ, Ouda JO, Lopez S, Nelson N, Omed H, Wallace RJ, Moss AR (2002): Propionate precursors as possible alternative electron acceptors to methane in ruminal fermentation. In: Takahashi J, Young BA (eds): Greenhouse Gases and Animal Agriculture. 1st edn. Elsevier Science, Amsterdam. 151–154.

Orskov ER, McDonald I (1979): The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science 92, 499–503.

Pandey P, Sirohi SK, Goel N, Mohini M (2012): Effect of addition of propionic acid on in vitro dry matter digestibility, methane production and rumen fermentation in mixed diets containing wheat straw with sorghum or berseem as roughage source. Wayamba Journal of Animal Science 4, 282–288.

Papatsiros VG, Katsoulos PD, Koutoulis KC, Karatzia M, Dedousi A, Christodoulopoulos G (2013): Alternatives to antibiotics for farm animals. CAB Reviews: Perspec-

https://doi.org/10.17221/100/2017-VETMED

tives in Agriculture, Veterinary Science, Nutrition and Natural Resources 8, doi: 10.1079/PAVSNNR20138032.

Sirohi SK, Pandey P, Goel N, Mohini M, Kundu SS (2012): Effect of tartaric acid addition on rumen fermentation, methane production and digestibility in different diets containing wheat straw in vitro. Online Journal of Animal and Feed Research 2, 308–313.

Tejido ML, Ranilla MJ, Garcia-Martinez R, Carro MD (2005): In vitro microbial growth and rumen fermentation of different substrates as affected by the addition of disodium malate. Journal of Animal Science 81, 31–38.

Van Soest PJ, Robertson JB, Lewis BA (1991): Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.

Veira DM (1986): The role of ciliate protozoa in nutrition of the ruminant. Journal of Animal Science 63, 1547–1560.

Received: May 25, 2017
Accepted after corrections: August 28, 2018