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Effects of a specific blend of essential oil on rumen degradability, total tract digestibility and fermentation characteristics in rumen fistulated cows

Abstract

Essential oils had been received much attention due to their antimicrobial properties against a wide range of microorganisms that manipulate rumen fermentation towards a better utilization of energy and protein. Six fistulated non-lactating Friesian dairy cows were used to investigate the effect of adding Crina® Ruminants (blend of essential oil) with 1g per cow per day to total mixed ration (grass silage, maize silage, soybean meal, rapeseed meal and wheat) of 7kg per day on the *in-situ* rumen dry matter degradability (ISDMD) and total tract digestibility using TiO_2 as marker. Fistulated cows were used as 3x2 Latin Square with factorial arrangement of treatment (with or without Crina® addition) in two periods. Each period extended for 45days (30days pre-experimental phase and 15days experimental phase). Ruminal fluid samples were collected to investigate the rumen fermentation parameters (ruminal pH value, volatile fatty acid (VFAs), and ammonia nitrogen (NH_3N) as well as acetate propionate ratio).

The results indicated that adding of Crina® to ruminant diet had significantly decreased ISDMD of grass silage and total mixed ration especially at long incubation time (12 and 48hours). The ISDMD and *in-situ* rumen crude protein degradability of soybean and rapeseed meal significantly increased due to Crina® addition. Crina® had no effect on total tract digestibility of dry matter(DM), organic matter(OM), crude protein(CP), starch, ether extract(EE), crude fiber(CF) and fiber fractions. Rumen fermentation parameters did not affected due to addition of Crina®. Results concluded that Crina® could limit the degradability of grass silage, increased degradability of soybean and rapeseed meal and had no effect on rumen fermentation characteristics.

Keywords: essential oil, crina® ruminants, rumen manipulation, rumen fermentation, *in-situ* method

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Introduction

Feed digestion in ruminant occurs mainly through the microbial fermentation in the rumen. Although rumen fermentation depredates plant fiber, starch and protein producing volatile fatty acids as a source of energy as well as microbial protein as a valuable source of digestible amino acids. Also, it has disadvantages and risks, known as rumen acidosis, losses of energy in form of methane and losses of protein in form of hyper-production of ammonia.[1] Increasing bypass starch and protein is important in highly producing dairy cows, as it is supports milk production more efficiently.[2] Therefore, it is important to manipulate rumen fermentation towards a better utilization of energy and protein. In the last few decades, feed additives such as antibiotics, ionophores, methane inhibitors and defaunating agents have been used to manipulate rumen fermentation. Recently, due to banning the use of antibiotic and the increasing in public concern over antibiotic residues and resistance, much effort has been devoted towards developing alternatives to antibiotics. Essential oil (EO) trend had gained an interest as a possible natural alternative antibiotic rumen fermentation modifiers.[3] Essential oils have received much attention[4,5] due to their antimicrobial properties against a wide range of microorganisms including bacteria, protozoa, and fungi.[6] McIntosh et al.[7] indicated that it is possible to use a blend of essential oil to manipulate rumen fermentation by selective suppression of certain microbial species that reduce protein degradation and promoting nitrogen escape from the rumen. Busquet et al.[8] found that essential oil affected rumen fermentation, reducing total volatile fatty acid (VFAs) with a linear increase in the molar proportion of propionate. Therefore, the objectives of this study were to investigate the effect of blend of essential oil supplementation to total mixed ration (TMR) on rumen synchronization (VFAs versus ammonia production and pH as indicator for those two processes) at short incubation times (1, 2, 3, 4, 5, 6, and 9h) and *in-situ* rumen degradation characteristics of dry matter of the used TMR and its individual components at long incubation times (12, 24, and 48h). Additionally, total tract digestibility of DM, OM, CP, starch and CF and CF fractions were determined using TiO_2 as a marker.

Materials and methods

Animals and diets

The present work was conducted at Chair of Animal Nutrition, Center of Life and Food Sciences, Weihenstephan, Technische Universität München, Germany. Six non-lactating Friesian dairy cows (live body weight approximately 650kg) were used to measure the

effect of blend of essential oil (Crina® Ruminants, DSM Nutritional Products Ltd. Basel, Switzerland) on the *in-situ* rumen degradation characteristics. The used product (Crina® Ruminants) was a mixture of different essential oils (Thymol, m-cresol, Guaiacol, Eugenol and Resorcinol). The cows were provided with rumen cannula (Bar Diamond Inc., Parma, Idaho, USA with 10cm internal width). During the *in-situ* experimental period, the cows were individually penned in a clean and air conditioned stall (temperature 20°C). Clean fresh water and salts blocks were offered for free choice. Daily dry matter intake was about 7.0kg and the cows were given the ration in two equal portions at 07.00 am and 04.00 pm. Each portion on DM basis was consisted of 2.24kg from grass silage, 0.5kg maize silage, 0.22kg soybean meal, 0.22kg rapeseed meal, 0.22kg wheat and 25g mineral and vitamin mixtures. The Crina® EO product was added individually every meal to the EO treated cows according to the company recommendation (0.5g per meal) as top dressing and thoroughly mixed to the other feed ingredients. Total mixed ration was given for 30days before start of the experiment for adaptation (pre-experimental phase) and extended throughout the experimental period (experimental phase). The chemical composition of the used feedstuffs is presented in Table 1. Fistulised cows were used as 3x2 Latin Square with factorial arrangement of treatment in two periods. During the first period 3 cows received control treatment (no EO) while the other animals were exposed to diet supplemented with EO. In the second period, the treatment was reversed thus providing each cow to serve as its own control. Each period lasted for 45days (30days pre-experimental phase and 15days experimental phase). The care, maintenance, handling and surgical techniques of the animals were carried out according to the guidelines of the German laws for animal care.

Table 1 Chemical composition of the different feedstuffs

Feedstuff	DM (%)	(%) DM								
		OM	CP	TL	NfE	CF	Hemi cell	Cellulose	Lignin	CA
TMR		91	17.5	3.95	44.1	25.4	17.7	21.9	2.79	8.73
Grass Silage	26.3	88.7	15.3	4.45	38.1	30.8	21.7	27.4	2.98	11.3
Maize Silage	37.1	96.9	6.6	3.75	61.1	25.4	18.6	20.8	1.97	3.12
Soybean	91.8	93.5	49.43	3.3	33.9	6.87	5	5.86	0.39	6.46
Rapeseed	89.2	93.2	36.5	3.4	36.4	16.9	8.94	12	7.64	6.77
Wheat	87.5	98.1	14.5	1.39	77.3	5.02	6.56	2.53	0.93	1.86

In-situ method

In this study *in-situ* DM degradability (%) of TMR and the individual components (grass silage, maize silage, soybean meal, rapeseed meal and wheat) without or with EO (0 or 1.0g per head per day) was studied using the nylon bag technique.[9] In contrast to common *in-situ* studies e.g. on protein degradability of distinct feed components added to the ration, the following study focused also on the impact of the EO additive on rumen fermentation kinetics of the entire ration. Another aspect was preparation of feed samples. Usually, the feed samples are dried and ground but this might modulate fermentation kinetics inside the nylon bag compared to the situation outside the bag. Therefore, the feed sample preparation was done with fresh materials. The bags (10 x 20cm) used in this study had a pore size of 53µm (R1020, Dohod Technology, Fairport, NY, USA). Four grams of DM (about 15g grass silage, 11g maize silage, 4.6g from each: soybean meal, rapeseed meal or wheat, as well as 12g from TMR) was weighed to the nearest 3 decimal points. The weighed materials were placed into previously labelled, dried (at 60°C for 48h) and weighed bags, which were incubated in the rumen of the fistulated cows. For TMR bags, the individual components were weighed and placed into the bags in the same proportion as present in the cows ration and thoroughly mixed. In order to guarantee homogeneous presence of EO in all tested material, EO was admixed to each of the nylon bags in the same proportion as it was present in the respective TMR and its components. Nylon bags of the control treatment received no EO addition. Twenty-four bags were prepared for each cow at each incubation point (4bags from each; grass silage, maize silage, soybean meal, rapeseed meal, wheat and TMR). Additionally, eighteen "0-hour" nylon bags were prepared (3bags for each treatment) to serve as control at each incubation time. Dry matter content of feed material used was determined for each incubation time. The test-bags were incubated in the rumen of the six cows just before the morning feeding at 07.00 a.m. for 1, 2, 3, 4, 5, 6, 9, 12, 24, and 48h. Bags were removed from the rumen (all in-all out system) and were immediately put into ice water to stop microbial activity. Then the bags were put together with the corresponding 0-hour bags into the washing tank with about 40L cold water and washed for about 5minutes and then washed in a washing machine (QUELLE WVA BASIC 74) for 19minutes. Afterwards the bags were freeze dried and weighed again to determine the *in-situ* rumen dry matter degradability (ISDMD%). In addition, the *in-situ* rumen crude protein degradability (%) was calculated for the incubation times 0, 1, 3, and 6hours.

Ruminal fluid samples collection

Ruminal fluid samples (about 200ml) were collected from each animal at the short term incubation periods (1, 2, 3, 4, 5, 6, and 9h) at the onset of incubation and at removal of nylon bags. Samples were divided into two portions; one portion was used to measure rumen pH directly and then centrifuged and frozen to be used for measure the ammonia nitrogen. The second portion was centrifugated and 10ml of the supernatant was preserved and frozen to determine VFAs later on.

Total tract digestibility

For measuring the total tract digestibility, the components of the concentrate (soybean, rapeseed and wheat) were pre-mixed in the ratio corresponding to the ratio in the actual total diet and the indigestible marker TiO_2 was added. The marker–concentrate mixture was thoroughly mixed with total ration with a final concentration of the marker of 0.1% on DM basis in the used TMR.

Chemical analysis

Samples of TMR (without and with addition of EO) were collected through the time course of the study, pooled, dried and ground, and submitted to chemical analysis of DM, crude ash, CP, total lipids (TL), crude fiber (CF), and fiber fractions (NDF, ADF, and ADL) according to the standard methods.[10] Rumen fluid pH value was immediately measured after sampling using a pH-meter (Schott, CG 842). Analysis of ammonia nitrogen was done by a modified method of Conway.[11] Determination of rumen juice VFAs (acetate, propionate, butyrate, valeric acid) was done according to the method of.[12] Lactate was analysed photometrically.

For determination of the *in-situ* rumen CP degradability (%) for the incubation times 0, 1, 3, and 6hours, the residue inside the bags after each incubation time for each feedstuff and for the same cow were collected and pooled and crude protein was determined used NIRS method. For determination of total tract digestibility, samples of TMR and faecal samples were collected at the last seven days within each experimental period, pooled, freeze-dried and grounded. CP, TL, crude ash, CF and fiber fractions (NDF, ADF, and ADL) as well as TiO_2 were analysed according to standard methods.[10,13,14]

Calculation

In-situ dry matter degradability or disappearance (ISDMD) of incubated material at a certain incubation interval was calculated as percentage of dry matter loss before and after incubation:

$$ISDMD\ (\%) = \frac{Weight\ before\ incubation\ (g)\ -\ Weight\ after\ incubation\ (g)}{Weight\ before\ incubation\ (g)} \times 100$$

Rumen dry matter degradation data were fitted to the exponential equation of.[9]

$$p = a + b(1 - e^{-c(t-t_0)})$$

for $t = t_0$

Where,

P = DM degradation (%) at time t

a=rapidly soluble fraction (%) b=insoluble but ruminally degradable (slowly degradable fraction) (%), c=constant rate of degradation of b (%/h), t_0 = lag time (h), defined as the time from beginning of incubation until beginning of degradation (delay time).

Effective rumen dry matter degradability (EDMD) was calculated following the equation of.[15]

$$P = a + \left[(b \times c)\,(c + k)\right] \times e^{-k \times t_0}$$

where, a, b, c and (t_0) are the same as in Calsamiglia S[1]

k (%h^{-1}) is the estimate rate of passage of the digesta from the rumen per hours.

The total tract digestibility was calculated as follows:

$$Total\ tract\ digestibility\ (\%) = 100 - \frac{(\%)\ indicator\ in\ feed}{(\%)\ indicator\ in\ feces} \times \frac{(\%)\ nutrient\ in\ feces}{(\%)\ nutrient\ in\ feed} \times 100$$

Statistical analysis

Average DM losses from bags within cows, treatment and incubation intervals as well as corresponding rumen fluid pH values were subjected to analysis of variance with GLM procedures of SAS.[16]

$$Y_{ij} = \mu + treatment_i + cow_j + e_{ij}$$

where,

Y_{ij} = observation value of the dependant variable

μ=overall mean
$treatment_i$=fixed effect of EO treatment
cow_j=fixed effect of cow (6 animals)
e_{ij} = residual error

Differences between treatment (EO addition: no vs. yes) were assessed for statistical significance by F-Test (treatment vs. e_{ij}) ($p < 0.05$).

The following tables show mean values of 6 cows treated either without (control) or with EO addition. The term "SE" denotes the residual error derived from analysis of variance. This provides an estimate about the biological variation of a parameter corrected for individual effects of cows and treatment. The term "P_{EO}" denotes the p-value of the treatment (with or without EO) derived from F-Test.

Results

In-situ rumen dry matter degradability of the different feedstuffs

In-situ rumen dry matter degradability (%) of the different feed ingredients with or without EO addition is presented in Table 2. In the current study the used feedstuffs were chosen to represent feed ingredients commonly used as source of fiber (grass silage and maize silage), protein (soybean meal and rapeseed meal) and starch (maize silage and wheat). There was no consistent and/or quantitatively relevant effect of EO addition on ISDMD of TMR or its individual components. *In-situ* rumen dry matter degradability of TMR after 12hours showed significantly ($p<0.05$) lower rumen dry matter degradability with EO addition when compared with the control diet (58.4 vs. 60.3%). Grass silage ISDMD at 12hours of incubation was significantly lower ($p<0.01$) with than without EO (52.0 vs. 54.1%) and continue to be the same ($p<0.02$) trend (76.0 vs. 77.6%) at 48hours of incubation. Maize silage degradability was high at the beginning of the incubation (52.7 vs. 51.4% with and without EO, respectively). Maize silage was increased slowly in degradability over the course of incubation until it reached 77.3 and 76.3 at 48hours of incubation for control and EO treatment, respectively. *In-situ* rumen dry matter degradability of protein source feedstuffs (soybean meal and rapeseed meal) at short incubation times (1-3hours for soybean meal and 2, 3 and 4hours for rapeseed meal) was significantly increased due to EO addition. On the other hand, no effect for EO on rumen degradability of soybean meal and rapeseed meal at long incubation time (12, 24 or 48hours of incubation). Rapeseed showed the lowest *in-situ* rumen dry matter degradability at the beginning of the incubation (29.6 and 27.4% for EO and control, respectively). Wheat as a cereal grain feedstuffs (high starch) showed the highest *in-situ* rumen degradability at the beginning of the incubation and EO significantly ($p<0.01$) increased the degradability (63.7 vs. 57.3% with and without EO, respectively). Wheat was almost completely degradable after 48hours (93.5% for both treatments).

In-situ rumen dry matter degradation kinetics of the different feedstuffs

In the present study, addition of EO product did not affect the

in-situ rumen dry matter degradation kinetics of the TMR and its individual components (Table 3).

The TMR rapidly soluble (a), slowly degradable fraction (b), the non degradable fraction (d), and the EDMD averaged 33.6, 49.6, 16.9, and 57.6%, respectively and they were not affected with EO addition. As grass silage constitute about 66% of the TMR, *In-situ* rumen dry matter degradation kinetics of grass silage was almost like TMR and did not affected with EO addition. However, the non degradable part of maize silage was much lower for the EO treatment than control (14.3 vs. 19.7%, respectively), the EDMD of maize silage did not affected (61.9 vs. 61.7% with and without EO, respectively). Due to the highly soluble fraction of maize silage between the all feedstuffs (mean 51.2%) the slowly degradable fraction was the lowest one (mean 31.8%). As a source of fiber (grass silage and maize silage), grain in maize silage gave it the higher EDMD than grass silage (61.8 vs. 52.1%). Soybean meal was completely degradable (non degradable fraction was 0.45%) and was the highest between the different feedstuffs in the slowly degradable fraction (averaged 66.8%). Rapeseed meal was the lowest in the rapidly degradable fraction (mean 26.2%). As a protein source, rapeseed meal was lower than soybean meal in the EDMD (57.8 vs. 69.5%, respectively). The EDMD of wheat was the height among the different feedstuffs (82.9 vs. 84.2% with and without EO, respectively).

Table 2 *In-situ* rumen dry matter degradability (%) of the different feedstuffs without (-) or with (+) addition of essential oils

Feedstuff	EO	Incubation time(h)										
		0	1	2	3	4	5	6	9	12	24	48
TMR	-	33.1	35.5	37.8	39.2	41.6	45.1	45.6	54.8	60.3[a]	72.6	80
	+	33.3	36.4	37.4	39.9	41.2	43.4	46.3	53.7	58.4[b]	70.7	79.3
	SE	-	0.99	1.35	1.49	1	1.28	2.83	3.13	1.26	2.24	1.52
	P_{EO}		0.17	0.66	0.44	0.51	0.07	0.68	0.55	0.05	0.2	0.46
Grass Silage	-	30.6	29.7[b]	31	31.6	34.5	38.1	39.1	46.2	54.1[a]	68.6	77.6[a]
	+	29.3	31.0[a]	31.5	32.8	34.1	35.7	38.4	47.4	52.0[b]	65.6	76.0[b]
	SE	-	0.8	0.8	0.82	1.09	3.15	1.88	3.04	0.85	2.07	0.83
	P_{EO}		0.04	0.34	0.05	0.52	0.26	0.55	0.52	0.01	0.05	0.02
Maize Silage	-	50.6	51.4	51.8	51.2	52.7	54.6	54.7	57.3	60.7	70.2	76.3
	+	51.7	52.7	52.5	52	52	53.1	55.7	58.9	60.6	68.9	77.3
	SE	-	1.56	0.6	3.61	1.9	2.5	3.58	3.4	2.87	2.7	1.46
	P_{EO}		0.21	0.09	0.72	0.56	0.37	0.66	0.44	0.94	0.44	0.29
Soybean	-	29.2	32.5[b]	35.8	37.9[b]	40.8	45.1	49.3	65.9	79.2	94.9	98.1
	+	31.1	33.8[a]	36.6	39.8[a]	42.3	46.9	50.8	67.5	77.9	91.7	98
	SE	-	0.59	0.69	0.48	1.46	1.64	3.16	3.58	4.9	3.01	0.22
	P_{EO}		0.01	0.11	0	0.14	0.11	0.46	0.46	0.66	0.13	0.48
Rapeseed	-	22.5	27.4	29.6[b]	32.2[b]	32.9[b]	36.3	40.7	53	65	79	82.3
	+	24.8	29.6	31.7[a]	34.8[a]	37.0[a]	38.8	43.5	56.1	64.4	76.9	80.9
	SE	-	1.78	1.05	0.44	1.69	1.88	2.4	2.88	3.3	1.59	1.31
	P_{EO}		0.08	0.02	0	0.01	0.07	0.1	0.12	0.78	0.07	0.14
Wheat	-	30.9	57.3[b]	68.5	70.9	77.1	79.3	80.7	85.6	90.3	92.5	93.5
	+	37.5	63.7[a]	70.5	76.1	76.6	80.1	84	89.6	89.7	92.5	93.5
	SE	-	2.38	5.25	5.28	2.85	3.78	7.2	7.09	1.72	2.69	1.39
	P_{EO}		0.01	0.55	0.15	0.79	0.73	0.47	0.37	0.58	0.99	0.94

"-", "+", control treatment, essential oil addition; SE, standard error (root MSE from 2 factorial analysis of variance); P_{EO}, p-value of the treatment; "0h" samples were analysed before incubation (no relevance of standard deviation), Means along the same column and feedstuff bearing different small letters are significantly different ($p<0.05$)

Table 3 Rumen degradability parameters and effective rumen dry matter degradability of the different feedstuffs with (+) or without (-) essential oil addition (bold values=estimated parameters, values below=standard deviation)

	d	**a**	**b**	**c**	**t_0**	**Passage rate (k, %/h)**
Feedstuff	**(%)**	**(%)**	**(%)**	**(%h^{-1})**	**(h)**	**6%**
TMR(-)	16.9	33.6	49.5	6.91	1.03	58.1
	±1.98	±0.65	±2.34	±1.74	±0.62	±1.75
TMR(+)	16.8	33.6	49.6	6.06	0.72	57.1
	±2.07	±0.78	±2.50	±1.55	±0.54	±2.27
Mean	16.9	33.6	49.6	6.49	0.88	57.6
Grass silage(-)	19.1	30.4	50.5	6.77	2.78	52.7
	±2.64	±0.25	±2.66	±1.66	±0.46	±1.71
Grass silage(+)	20.2	30.4	49.5	6.39	2.64	51.4
	±3.48	±0.87	±4.15	±2.05	±1.25	±1.90
Mean	19.7	30.4	50	6.58	2.71	52.1
Maize silage(-)	19.7	50.4	29.9	4.96	2.67	61.7
	±2.92	±0.71	±2.57	±1.39	±3.17	±2.07
Maize silage(+)	14.3	52	33.7	3.91	3.6	61.9
	±6.08	±0.29	±6.06	±1.75	±1.02	±1.92
Mean	17	51.2	31.8	4.44	3.14	61.8
Soybean-M(-)	0.1	32.2	67.6	11.4	2.75	69.6
	±0.19	±1.13	±1.26	±2.02	±0.83	±2.60
Soybean-M(+)	0.8	33.3	65.9	10.6	2.37	69.4
	±1.22	±0.63	±1.46	±2.30	±0.43	±3.33
Mean	0.45	32.75	66.8	11	2.56	69.5
Rapeseed-M(-)	15	25.4	59.6	10.7	2.35	57.5
	±3.18	±2.67	±5.54	±3.70	±1.60	±1.81
Rapeseed-M(+)	16.8	26.9	56.3	10.2	1.6	58
	±2.17	±2.01	±3.96	±3.69	±1.20	±2.54
Mean	15.9	26.15	58	10.45	1.98	57.8
Wheat(-)	8.8	35.4	55.7	36.7	0	82.9
	±1.63	±4.52	±5.60	±12.4	±0.00	±2.59
Wheat(+)	9	41.1	49.9	43	0	84.2
	±0.54	±1.71	±1.81	±19.7	±0.00	±2.37
Mean	8.9	38.25	52.8	39.85	0	83.6

d=Non degradable fraction (%), a=rapidly soluble fraction (%), b=insoluble but ruminally degradable (slowly degradable fraction) (%), c=constant rate of degradation of b (%/h), t_0=lag time (h), defined as the time from beginning of incubation until beginning of degradation (delay time)

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In-situ rumen crude protein degradability of the different feedstuffs

In-situ rumen crude protein degradability(%) of the different feed ingredients with or without EO addition is presented in Table 4. Addition of EO had no consistent and/or quantitatively relevant effect on *in-situ* rumen crude protein degradability(%) of TMR, grass silage and maize silage. On the other hand addition of EO significantly ($p<0.007$) increased *in-situ* rumen crude protein degradability (%) of soybean meal after one hour of incubation (16.5 vs. 13.6%). As well, EO significantly increased *in-situ* rumen crude protein degradability (%) of rapeseed meal after one hour (25.9 vs. 20.3%), three hours (34.8 vs.28.9%), and six hour of incubation (45.3 vs. 39.3%). Addition of EO had numerically but not significantly increased *in-situ* rumen crude protein degradability(%) of wheat. Those results indicated that addition of EO had a great tendency to increase crude protein degradability especially with feedstuffs rich in protein (soybean meal and rapeseed meal).

Rumen fermentation characteristics

Rumen fermentation characteristics and physiological parameters (rumen pH value, ammonia nitrogen, acetic acid, propionic acid, butyric acid, total volatile fatty acids and its molar proportion) are illustrated in Table 5. Addition of EO had no significant effect on rumen fermentation characteristics. The roughage concentrate ratio of the current diet was 80 to 20%, respectively. Therefore, rumen fluid pH was typical for this diet as it start with pH averaged value of 6.8 just before feeding at 7.00 (6.85 vs. 6.74 with and without EO, respectively). Rumen pH reached the lowest value after 3hours of feeding at 10.00 am to reach averaged value of 6.54 (6.56 vs. 6.53 with and without EO, respectively). Afterwards rumen pH increased again and reached the highest value after 9 hours of feeding at 16.00 pm to reach averaged value of 6.73 (6.75 vs. 6.72 with and without EO, respectively). Rumen fluid ammonia nitrogen and total VFAs as an indication of rumen fluid pH, did not affected by EO addition. Addition of EO had no significant effect on molar proportion of VFAs (acetate to propionate ratio) and it was indicator of high roughage diet of the current study (80%).

Total tract digestibility using TiO_2

The effects of EO supplementation on total tract digestibility (%) are presented in Table 6. The obtained results indicated that addition of EO had no significant effect on total tract digestibility of dry matter (80 vs. 71.3), organic matter (77.5 vs. 77.8), crude protein (75.2 vs. 75) and crude fiber (77.5 vs. 79.1).

Discussion

Due to the increase in public concern over antibiotic residues and resistance, essential oil trend had gained an interest as a possible natural alternative antibiotic rumen fermentation modifiers.[3] Therefore, the aim of the present work was to investigate whether dietary addition of a specific mixture of EO compound (Crina® Ruminant) could affect rumen fermentation characteristics, *in-situ* rumen dry matter degradability and total tract digestibility in fistulated cattle fed a maintenance diet of TMR (7kg) with a roughage concentrate ratio of 80 to 20%, respectively. As rumen is the main chamber for digesting feed DM and fiber. Therefore, ruminal DM and fiber digestibility are important indices in evaluating the effects of EO on ruminant feed digestibility.[8,17] In the current study, grass silage and maize silage were used as a source of fiber. There was no consistent and/or quantitatively relevant effect of EO addition on *in-situ* dry matter degradability of TMR and grass silage as the main component of the current TMR (65% grass silage). *In-situ* rumen dry matter degradability of TMR and grass silage after 12 and 48hours of incubation showed significantly lower rumen dry matter degradability with EO addition than control diet. As grass silage contain high level of fiber and the *in-situ* rumen degradability of fiber did not determine in this study, this results might indicate that addition of EO (1g per head per day) tend to have a negative effect on fiber degradability. This results are in agree with Tager & Krause,[18] Lin et al.[19] They found that high levels of EO negatively affect ruminal fiber degradation. However total bacteria was not determine in the current work, the negative effect of EO on fiber digestion might be attributed to the inhibition of total ruminal bacteria (cellulytic bacteria); a similar observation was also found in other *in vivo* studies Lin et al.,[19] Soltan et al.,[20] Santos et al.,[21] Patra & Yu.[22] This results are disagree with Benchaar et al.,[23] who observed no effect of EO supplementation on counts of ruminal cellulolytic bacteria in dairy cows. Soybean meal and rapeseed meal as a protein feedstuffs showed higher rumen DM degradability at short incubation times as influenced by EO supplementation. This DM degradation was confirmed with higher rumen CP degradability (Table 4) especially with rapeseed meal. The higher CP degradability of soybean and rapeseed meal is unclear and could be attributed to activation of proteolytic bacteria due to addition of EO. This results disagree with the previous studies which pointed to decrease in crude protean degradability. McIntosh et al.[7] observed that a species known as hyperammonia-producing bacteria were inhibited by Crina® EO and suggested that the main effect of EO might occurred during the final phase of protein degradation or no change in crude protein degradation in growing heifers[24] and in sheep[25] supplemented with 700 and 110mg of Crina® EO, respectively.

However, obtained results indicated that addition of EO had negative effect on rumen fiber digestion, it had no effect on total tract digestibility (Table 6) of dry matter (80 vs. 71.3), organic matter (77.5 vs. 77.8), crude protein (75.2 vs. 75) and crude fiber (77.5 vs. 79.1). Therefore, the apparent digestibility is a rough index and is usually not sufficient to evaluate effects of EO on nutrient digestion of ruminant digestive tract, and therefore, measurement of ruminal or intestinal digestibility is necessary.[19] This would agree with the results of Castillejos et al.,[26] who observed no change in DM, OM, NDF, and CP digestibility when a Crina EO mixture was added at the dose of 3.8mg/L of ruminal fluid in continuous-culture fermenters. As well Benchaar et al.[27] found no difference in DM, OM, NDF and CP digestibility when essential oil was added to lactating dairy cow (2g per head per day). This would be explained by improved NDF and ADF digestibility[19] or ADF alone[27] post rumen because EO can compensate for the negative effects.

The current study showed that EO had no effect on rumen fermentation characteristics (rumen pH, rumen volatile fatty acids and its molar proportion of acetate to propionate, rumen ammonia nitrogen). Rumen pH value as indicator of rumen volatile fatty acids and rumen ammonia nitrogen did not change. These result agree with Newbold et al.,[25] Castillejos et al.,[26] Meyer et al.,[28] and Giannenas et al.,[29] they did not find any differences in rumen pH when EO mixtures were administered in the diets of dairy cows. On the other hand, Benchaar et al.[4,30] reported a slight but not significantly increase in rumen pH values in dairy cows when supplementing with EO mixture.

Previously studies by Castillejos et al.,[1] Benchaar et al.,[4] Benchaar et al.,[23] Newbold et al.,[25] Beauchemin & McGinn,[27] found that EO

had no effects on the ruminal total VFAs concentrations and on molar proportions of individual VFAs. On the other hand, Castillejos et al.[26] reported an increase in total VFAs concentrations and no change in molar proportions of individual VFAs when Crina® EO was added to continuous culture fermenters. Contrarily, Busquet et al.[8] and Varga et al.,[31] found that EO affected rumen fermentation, reducing total VFAs with a linear increase in the molar proportion of propionate.

The present study indicated that the addition of EO had no effect on ruminal fluid concentration of ammonia nitrogen. This would agree with the results of Castillejos et al.,[26] and Busquet et al.,[32] who reported that EO had no effect on ammonia nitrogen concentration in continuous-culture fermenters. Benchaar et al.[23] observed no effect of EO on ammonia nitrogen concentration in the rumen of lactating cows fed silage-based diets. On the other hand, McIntosh et al.,[7] Newbold et al.,[25] observed a reduction in the rate of ammonia nitrogen production when cows and sheep fed 1g and 100mg/d of Crina® EO, respectively. McIntosh et al.[7] suggesting that Crina® EO reduced ammonia production in ruminal fluid by inhibiting the activity of hyperammonia- producing bacteria, that characterized by high deaminative activity and as being responsible for a significant proportion of ammonia produced in rumen.

This discrepancy between the different EO studies could be due to the diet used (high or low concentrate in diet), the procedure used (*in vivo* or *in vitro*), dose of the EO and the length of exposure of ruminal bacteria to EO.[30] Busquet et al.[32] and Cardozo et al.,[33] found that the effects of different EO on rumen microbial fermentation lost after 6 days of incubation in a continuous culture system which indicate that ruminal bacteria could adapt to EO after period. Results from *in vitro* studies[8,32,33] found that EO are effective on the activities of ruminal bacteria at high doses but at lower doses (1g/cow per day), EO have little or no effect on rumen microbial fermentation. Therefore, the variable effects of EO on rumen microbial fermentation could be explained by the different doses used. Therefore, longer term *in vivo* studies with diets different in roughage concentrate ratio are required to clearly establish the effects of EO supplementation at high feeding doses.

Table 4 *In-situ* rumen crude protein degradability (%) of the different feedstuffs without (-) or with (+) addition of essential oils

Feedstuff	EO	Incubation time(h)			
		0	1	3	6
TMR	-	44.77	48.32	53.73	58.93
	+	47.53	46.37	56.25	59.01
	SE	-	2.25	2.82	3.26
	P_{EO}	-	0.194	0.182	0.969
Grass Silage	-	58.59	55.44	57.39	62.26
	+	56.49	60.68	60.18	61.64
	SE	-	7.23	2.29	1.42
	P_{EO}	-	0.264	0.088	0.483
Maize Silage	-	68.66	71.85	69.74	72.62
	+	67.24	72.23	71.73	70.79
	SE	-	2.33	3.27	5.06
	P_{EO}	-	0.786	0.338	0.559
Soybean-M	-	9.54	13.58^{a}	22.57	34.26
	+	12.29	16.49^{b}	25.07	37.4
	SE	-	1.15	2.03	2.84
	P_{EO}	-	0.007	0.086	0.113
Rapeseed-M	-	8.24	20.26^{a}	28.93^{a}	39.27^{a}
	+	15.22	25.91^{b}	34.77^{b}	45.31^{b}
	SE	-	2.13	1.36	1.87
	P_{EO}	-	0.006	0.001	0.003
Wheat	-	22.74	35.75	55.17	78.42
	+	22.91	39.57	63.77	80.21
	SE	-	6.9	6.57	8.13
	P_{EO}	-	0.381	0.073	0.719

"-", "+", control treatment, essential oil addition; SE, standard error (root MSE from 2 factorial analysis of variance); P_{EO}, p-value of the treatment; "0h" samples were analysed before incubation (no relevance of standard deviation), Means along the same column and feedstuff bearing different small letters are significantly different ($p<0.05$)

Citation: Metwally A, Deml M, Fahn C, et al. Effects of a specific blend of essential oil on rumen degradability, total tract digestibility and fermentation characteristics in rumen fistulated cows. *J Dairy Vet Anim Res.* 2016;3(2):51–60. DOI: 10.15406/jdvar.2016.03.00072

Table 5 Rumen fluid pH value, ammonia nitrogen and volatile fatty acids (mg/l) of the different cattle with (+) or without (-) essential oil product addition

	Time							
EO		**7:00**	**8:00**	**9:00**	**10:00**	**11:00**	**12:00**	**13:00**	**16:00**
Rumen pH	-	6.74	6.65	6.65	6.53	6.55	6.65	6.69	6.72
	+	6.85	6.6	6.7	6.56	6.67	6.74	6.63	6.75
	SE	0.12	0.12	0.07	0.13	0.09	0.21	0.12	0.17
	P_{EO}	0.17	0.53	0.29	0.77	0.08	0.49	0.45	0.82
NH_3-N	-	65.9	392.9	236.8	380.6	320	107.9	85.6	29.8
	+	88.2	407.9	231.8	401.9	247.5	101.2	93.5	33.3
	SE	17.3	282.9	52.3	256.3	213.2	38.9	22.6	15.9
	P_{EO}	0.08	0.93	0.88	0.89	0.58	0.78	0.57	0.72
Acetic acid	-	4.43	4.71	4.51	5.1	4.58	4.45	3.85	4.06
	+	4.15	4.84	3.86	4.98	4.51	4.15	3.91	4.18
	SE	1.36	0.83	0.93	1.28	1.28	1.46	1.19	1.12
	P_{EO}	0.74	0.81	0.28	0.88	0.93	0.74	0.93	0.86
Propionic acid	-	1.22	2.04	1.9	2	1.69	1.56	1.21	1.16
	+	1.09	2.14	1.56	1.97	1.56	1.41	1.22	1.18
	SE	0.46	0.42	0.35	0.63	0.46	0.64	0.4	0.37
	P_{EO}	0.64	0.69	0.15	0.92	0.64	0.7	0.97	0.95
Butyric acid	-	1	1.8	1.77_a	1.87	1.67	1.52	1.23	1.06
	+	0.94	1.91	1.37_b	1.88	1.55	1.3	1.25	1.09
	SE	0.32	0.26	0.23	0.5	0.37	0.49	0.36	0.32
	P_{EO}	0.78	0.49	0.03	0.96	0.59	0.47	0.9	0.9
Valeric acid	-	0.26	0.41	0.52	0.59	0.55	0.45	0.35	0.27
	+	0.26	0.4	0.41	0.62	0.51	0.4	0.36	0.3
	SE	0.09	0.06	0.07	0.12	0.13	0.18	0.12	0.08
	P_{EO}	0.95	0.75	0.06	0.66	0.56	0.68	0.89	0.53
Total VFAs	-	6.91	8.96	8.7	9.56	8.5	7.99	6.64	6.56
	+	6.44	9.28	7.2	9.45	8.13	7.27	6.75	6.75
	SE	2.22	1.54	1.52	2.51	2.23	2.76	2.06	1.89
	P_{EO}	0.73	0.73	0.15	0.94	0.79	0.67	0.93	0.87
Ace/Pro ratio	-	3.75	2.31	2.37	2.56	2.76	2.93	3.2	3.54
	+	3.84	2.28	2.51	2.56	2.88	2.98	3.2	3.58
	SE	0.37	0.09	0.34	0.18	0.19	0.23	0.13	0.17
	P_{EO}	0.66	0.6	0.51	0.98	0.32	0.71	0.93	0.75
Lactic acid	-	0.008	0.584	0.137	0.014	0.007	0.008	0.012	0.006
	+	0.011	0.703	0.067	0.006	0.01	0.006	0.013	0.005
	SE	0.007	0.351	0.194	0.011	0.01	0.003	0.011	0.004
	P_{EO}	0.56	0.584	0.556	0.28	0.556	0.293	0.856	0.655

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Table 6 Total tract digestibility of the different cattle with (+) or without (-) essential oil product addition

	Digestibility(%)										
EO	**DM**	**OM**	**CP**	**TL**	**CF**	**NfE**	**Hemicell**	**Cell.**	**Lignin**	**CA**	**Energy**
-	80	77.5	75.2	69.7	77.5	79.1	83.3	81.4	13.6	27.9	76.8
+	71.3	77.8	75	69.2	79.1	78.8	83.7	82.1	13.7	28	77.1
SE	5.15	1.16	1.46	2.11	1.54	1.43	5.06	3.88	21.25	14.43	1.54
P_{EO}	0.91	0.69	0.84	0.69	0.13	0.77	0.89	0.76	1	0.99	0.78

SE, standard error (root mean square error from 2-way ANOVA); P_{EO}, p-value of the treatment

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Conflict of interest

Author declares that there is no conflict of interest.

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