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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

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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 1 g per cow per day to total mixed ration (grass silage, maize silage, soybean meal, rapeseed meal and wheat) of 7 kg per day on the *in-situ* dry matter degradability (ISDMD) and total tract digestibility using TiO_2 as marker. Fistulated cows were used in a 3x2 Latin Square with factorial arrangement of treatment (with or without Crina® addition) in two periods. Each period extended for 41 days (30 days pre-experimental phase and 11 days experimental phase). Ruminal fluid samples were collected to investigate the rumen fermentation parameters (ruminal pH value, volatile fatty acids (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 48 hours). 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 were 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.

Key words: Essential oil · Crina® ruminants · Rumen manipulation · Rumen fermentation · *In-situ* method

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 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

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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 (volatile fatty acids "VFAs" versus ammonia production and pH as indicator for those two processes) at short incubation times (1, 2, 3, 4, 5, 6 and 9 h) and *in-situ* rumen degradation characteristics of dry matter of the used TMR and its individual components at long incubation times (12, 24 and 48 h). Additionally, total tract digestibility of dry matter (DM), organic matter (OM), crude protein (CP), starch and crude fiber (CF) and crude fiber 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 650 kg) 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 10 cm 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 7.0 kg 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 2.24 kg from grass silage, 0.5 kg maize silage, 0.22 kg soybean meal, 0.22 kg rapeseed meal, 0.22 kg wheat and 25 g mineral and vitamin mixtures. The Crina® EO product was added individually every meal to the EO treated cows according to the company recommendation (0.5 g per meal) as top dressing and thoroughly mixed to the other feed ingredients. Total mixed ration was given for 30 days before start of the experiment for adaptation (pre-experimental phase) and extended throughout the experimental period (experimental phase, 11 day). The chemical composition of the used feedstuffs is presented in Table 1. Fistulated cows were used in a 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 41 days (30 days pre-experimental phase and 11 days 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.

***In-situ* Method:** In this study *in-situ* rumen DM degradability (%) of TMR and the individual components (grass silage, maize silage, soybean meal, rapeseed meal and wheat) without or with EO (0 or 1.0 g 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 20 cm) used in this study had a pore size of 53 µm (R1020, Dohod Technology, Fairport, NY, USA). Four grams of DM (about 15 g grass silage, 11 g maize silage, 4.6 g from each: soybean meal, rapeseed meal or wheat, as well as 12 g from TMR) was weighed to the nearest 3 decimal points. The weighed materials were placed into previously labelled, dried (at 60°C for 48 h) 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 (4 bags from each; grass silage, maize silage, soybean meal, rapeseed meal, wheat and TMR). Additionally, eighteen "0-hour" nylon bags were prepared (3 bags 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 48 h. Bags were removed from the rumen (all in – all out system) and were immediately put into ice

Table 1: Chemical composition of the different feedstuffs

		(%) DM								
Feedstuff	DM (%)	OM	CP	TL	NfE	CF	Hemicell.	Cellulose	Lignin	Ash
TMR		91.04	17.50	3.95	44.1	25.4	17.7	22.0	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	37.09	96.9	6.60	3.75	61.11	25.42	18.62	20.80	1.97	3.12
Soybean	91.8	93.5	49.43	3.30	33.94	6.87	5.00	5.86	0.39	6.46
Rapeseed	89.2	93.2	36.51	3.40	36.37	16.95	8.94	12.00	7.64	6.77
Wheat	87.5	98.1	14.46	1.39	77.26	5.02	6.56	2.53	0.93	1.86

DM, dry matter; OM, organic matter; CP, crude protein; TL, total lipid; NFE, nitrogen free extract; CF, crude fiber.

water to stop microbial activity. Then the bags were put together with the corresponding 0-hour bags into the washing tank with about 40 L cold water and washed for about 5 minutes and then washed in a washing machine (QUELLE WVA BASIC 74) for 19 minutes. 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 6 hours.

Ruminal Fluid Samples Collection: Ruminal fluid samples (about 200 ml) were collected from each animal at the short term incubation periods (1, 2, 3, 4, 5, 6 and 9 h) 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 centrifuged and 10 ml 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), 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 Geissler *et al.* [12]. Lactate was analysed photometrically.

For determination of the *in-situ* rumen CP degradability (%) for the incubation times 0, 1, 3 and 6 hours, 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 and 14].

Calculation: *In-situ* rumen 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:

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

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

$$[1]\ p = a + b(1 - e^{-c(t-t)}) \text{ for } t = t_0$$

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 McDonald [15].

$$P = a + \left[(b \times c)/(c + k)\right] \times e^{-k \times t_0} \quad (2)$$

where, a, b, c and (t_0) are the same as in equation [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:

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

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].

$$Y_{ij} = \mu + \text{treatment}_i + \text{cow}_j + e_{ij}$$

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 12 hours 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 12 hours 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 48 hours 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 48 hours 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 (1-3 hours for soybean meal and 2, 3 and 4 hours 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 48 hours of incubation). Rapeseed showed the lowest *in-situ* 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 48 hours (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 %,

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

		Incubation time (h)										
Feedstuff	EO	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	80.0
	+	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	1.00	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	0.20	0.46
Grass silage	-	30.6	29.7[b]	31.0	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	-	0.80	0.80	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	52.0	52.0	53.1	55.7	58.9	60.6	68.9	77.3
	SE	-	1.56	0.60	3.61	1.90	2.50	3.58	3.40	2.87	2.70	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	98.0
	SE	-	0.59	0.69	0.48	1.46	1.64	3.16	3.58	4.90	3.01	0.22
	P_{EO}		0.01	0.11	0.00	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	53.0	65.0	79.0	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	2.40	2.88	3.30	1.59	1.31
	P_{EO}		0.08	0.02	0.00	0.01	0.07	0.10	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	84.0	89.6	89.7	92.5	93.5
	SE	-	2.38	5.25	5.28	2.85	3.78	7.20	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 (Mean ± SD)

Feedstuff	d (%)	a (%)	b (%)	c (%h^{-1})	t_0 (h)	Passage rate (k, %/h) 6%
TMR (-)	16.9 ±1.98	33.6 ±0.65	49.5 ±2.34	6.91 ±1.74	1.03 ±0.62	58.1±1.75
TMR (+)	16.8 ±2.07	33.6 ±0.78	49.6 ±2.50	6.06 ±1.55	0.72 ±0.54	57.1±2.27
Mean	16.9	33.6	49.6	6.49	0.88	57.6
Grass silage (-)	19.1 ±2.64	30.4 ±0.25	50.5 ±2.66	6.77 ±1.66	2.78 ±0.46	52.7±1.71
Grass silage (+)	20.2 ±3.48	30.4 ±0.87	49.5 ±4.15	6.39 ±2.05	2.64 ±1.25	51.4±1.90
Mean	19.7	30.4	50.0	6.58	2.71	52.1
Maize silage (-)	19.7 ±2.92	50.4 ±0.71	29.9 ±2.57	4.96 ±1.39	2.67 ±3.17	61.7±2.07
Maize silage (+)	14.3 ±6.08	52 ±0.29	33.7 ±6.06	3.91 ±1.75	3.60 ±1.02	61.9±1.92
Mean	17.0	51.2	31.8	4.44	3.14	61.8
Soybean (-)	0.10 ±0.19	32.2 ±1.13	67.6 ±1.26	11.4 ±2.02	2.75 ±0.83	69.6±2.60
Soybean (+)	0.80 ±1.22	33.3 ±0.63	65.9 ±1.46	10.6 ±2.30	2.37 ±0.43	69.4±3.33
Mean	0.45	32.75	66.8	11.00	2.56	69.5
Rapeseed (-)	15.0 ±3.18	25.4 ±2.67	59.6 ±5.54	10.7 ±3.70	2.35 ±1.60	57.5±1.81
Rapeseed (+)	16.8 ±2.17	26.9 ±2.01	56.3 ±3.96	10.2 ±3.69	1.6.0 ±1.20	58.0±2.54
Mean	15.9	26.15	58.0	10.45	1.98	57.8
Wheat (-)	8.8 ±1.63	35.4 ±4.52	55.7 ±5.60	36.7 ±12.4	0.0 ±0.00	82.9±2.59
Wheat (+)	9.0 ±0.54	41.1 ±1.71	49.9 ±1.81	43.0 ±19.7	0.0 ±0.00	84.2±2.37
Mean	8.90	38.25	52.8	39.85	0.00	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).

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

		Incubation time (h)			
Feedstuff	EO	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	-	9.54	13.58[a]	22.57	34.26
	+	12.29	16.49[b]	25.07	37.40
	SE	-	1.15	2.03	2.84
	P_{EO}	-	0.007	0.086	0.113
Rapeseed	-	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	-	6.90	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 ($p < 0.05$)

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 was 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 was 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 highest among the different feedstuffs (82.9 vs. 84.2% with and without EO, respectively).

***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

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	6.60	6.70	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
NH3-N	-	65.9	392.9	236.8	380.6	320.0	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	2.0	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	-	1.00	1.80	1.77^{a}	1.87	1.67	1.52	1.23	1.06
	+	0.94	1.91	1.37^{b}	1.88	1.55	1.30	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 VFA	-	6.91	8.96	8.7	9.56	8.50	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

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 MJ ME
-	80.0	77.5	75.2	69.7	77.5	79.1	83.3	81.4	13.6	27.9	76.8
+	71.3	77.8	75.0	69.2	79.1	78.8	83.7	82.1	13.7	28.0	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	1.00	0.99	0.78

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 3 hours 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, were 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 (7 kg) 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 48 hours of incubation showed significantly lower rumen dry matter degradability with EO addition than control diet. As grass silage contains high level of fiber and the *in-situ* rumen degradability of fiber was not determined in this study, these results might indicate that addition of EO (1 g per head per day) tend to have a negative effect on fiber degradability. These results are in agreement with Tager and Krause [18] and Lin *et al*. [19]. They found that high levels of EO negatively affect ruminal fiber degradation. However total bacteria was not determined 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] and Patra and Yu, [22]. These results 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 110 mg 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 3.8 mg/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 (2 g 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 significant 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] and Beauchemin and 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] and Newbold *et al*. [25] observed a reduction in the rate of ammonia nitrogen production when cows and sheep fed 1 g and 100 mg/d of Crina® EO, respectively. McIntosh *et al*. [7] suggested that Crina® EO reduced ammonia production in ruminal fluid by inhibiting the activity of hyperammonia- producing bacteria, that are 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 were 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 is effective on the activities of ruminal bacteria at high doses but at lower doses (1 g/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.

CONCLUSIONS

From the current study, we can conclude that Crina® could limit the degradability of grass silage, increased degradability of soybean and rapeseed meal and had no effect on rumen fermentation characteristics.

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