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Effects of Different Oils on the Fatty Acid Profiles of Culture Medium and Ruminal Microorganisms *in vitro*

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Abstract: This study was carried out to characterize the effects of different oils on fatty acid profiles in both culture medium and ruminal microbes *in vitro* by 4 goats. Four treatments were peanut oil, rapeseed oil, corn oil and soybean oil, respectively. The results showed that significant differences were found in all kinds of fatty acids identified between treatment oils ($p<0.05$). The percentage of stearic acid (average of 57.46%), palmitic acid (average of 16.50%) and oleic acid (average of 10.77%) was comparatively higher in 24 h culture medium regardless of treatments and there were profound effects of oils on the fatty acid composition of 24 h culture medium and most kinds of fatty acids in culture medium were related to these in substrate oils (2-tailed <0.05). Additionally, regardless of treatments stearic acid (average of 36.09%), palmitic acid (average of 27.37%) and oleic acid (average of 18.05%) were most abundant in microbes. However, significant relationships were only found in few kinds of fatty acids between microbes and substrate oils (2-tailed <0.05). Taken together, fatty acid profiles of oils had significant effects on that of 24 h culture medium while no remarkable effects on that of ruminal microorganisms *in vitro*.

Key words: Culture medium, fatty acid profiles, oil, rumen microbes, oleic acid, goats

INTRODUCTION

Fatty Acids (FA) are important as a source of energy as structural components of cell membranes as signaling molecules and as precursors for the synthesis of eicosanoids (Calder, 2003, 2005, 2006). Thus, they have considerable impact on immune function and health (Calder, 2001; Yaqoob, 2003; Shaikh and Edidin, 2006). The rumen is the main site of microbial digestion and harbors a complex array of different microbes which act synergistically to break down feed for the host animal. That means that the rumen is a link between host animal and feedstuff (Zhu, 2004).

Feeds with high lipid content for ruminants are of interest for several reasons. Firstly, their high-energy density makes them an attractive supplement. Secondly, oils can alter rumen fermentation and metabolism inhibit methanogenesis and decrease the CH4:VFA ratio (Busquet *et al*., 2005; Jalc *et al*., 2009; Kongmun *et al*., 2010).

Thirdly, they afford a means of producing animal products containing fat with a composition which coincides with current consumer preferences hence, the recent interest in increasing the polyunsaturated fatty acids or Conjugated Linoleic Acid (CLA) in milk fat (Loor *et al*., 2002; Bu *et al*., 2007). Considering the impacts of ruminal microbe on dairy production in their fatty acid profiles, recently several studies about the fatty acid compositions of rumen microbes have been published (Or-Rashid *et al*., 2007, 2009; Sultana *et al*., 2011). It is however that researches related to the effect of oils with various FA profiles on ruminal microbes are still few. The current research was conducted to characterize the relationship between oil type and culture medium or ruminal organism on their fatty acid profiles *in vitro*.

MATERIALS AND METHODS

All procedures involving animals were approved by the Yangzhou University Animal Care and Use Committee of the Jiangsu province, China.

Experimental animal and treatments design: This research was carried out from July to September 2011. Four Xuhuai White goats fitted with rumen cannulas were selected from the Experimental Farm of Yangzhou University (Yangzhou, Jiangsu province, P.R. China) and were used as donors of rumen fluid.

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They were ca, 1.5 years of age and weighed on average 29.7±1.4 kg live weight. The animals were offered forage grass daily throughout the experimental period and were fed twice a day at 700 and 1900 h. They also had free access to clean drinking water. The treatments included peanut oil (Laiyang Luhua Aromatic Peanut Oil Co., Ltd, Laiyang, Shandong province, China, Cat. No: ECS004202) as Group A, rapeseed oil (Anhui Shuangfu Grain and Oil Co., Ltd., Chaohu, Aihui province, China) as Group B, corn oil (Qingdao Jiali Vegetable Oil Co., Ltd., Qingdao, Shandong province, China, Cat. No: 157112) as Group C and soybean oil (Shanghai Sea Lions Oil Industry Co., Ltd., Shanghai, China, Cat. No: 157099) as Group D. Treatments were run in triplicate and a set of appropriate blank (without substrate) was included. Composition of *in vitro* culture substrates was shown in Table 1.

Iodine value of substrate oils testing: Iodine value of substrate oils were monitored following the method described as vegetable oils-determination of iodine value (China National Standard Method: GB/T 5532-1995).

Rumen fluid collection and *in vitro* culture: The ruminal fluid was collected before the morning feeding and was kept temperate and anaerobic using a thermos which was pre-heated and pre-fluxed of carbon dioxide (CO_2) while being transported. Before being mixed with pre-heated reduced Menke buffer (Menke and Steingass, 1988), the ruminal fluid was strained through four layers of gauze (1000 im pore size, MedPro Novamed AG, Flawil, Switzerland). The ruminal fluid/buffer mixture (1:2, v/v; further on called incubation medium) incubation medium was placed in a water-bath maintained at 39°C with continuous flux of CO_2 and mixed for ca. 15 min. Then, incubation medium was dispensed into pre-warmed incubation flasks already containing the respective experimental treatments. About 1.5 g of each treatment substrate was weighed accurately and incubated with 150 mL of incubation medium for 24 h.

Culture medium and rumen microbes sampling: The entire contents of each culture were removed after 24 h culture and centrifuged at 500×g for 15 min. Then, the supernatant fluid was collected and centrifuged again at 15,000×g for 10 min. The resulting supernatant solution was removed as culture medium for subsequent examination. The solid residue was also collected as rumen microbes and intended for subsequent examination after vacuum freeze-drying.

Table 1: Composition of *in vitro* culture substrates

Composition (%)	A (Peanut oil)	B (Rapeseed oil)	C (Corn oil)	D (Soybean oil)
Starch	19	19	19	19
Casein	10	10	10	10
Cellulose	67	67	67	67
Peanut oil	4	-	-	-
Rapeseed oil	-	4	-	-
Corn oil	-	-	4	-
Soybean oil	-	-	-	4
Total	100	100	100	100

Determination of fatty acid profiles

Oil sample preparation: About 2 mL of each treatment oils was taken and evaporated under nitrogen gas until dryness at 45°C in a rotary evaporator. Then, 2 mL of 10% sulfuric acid in methanol (H_2SO_4-CH_3OH) (was added into each oil sample tube, capped and placed in a water bath for 20 min at 70°C. Subsequently, 1 mL deionized water and 2 mL hexane was added into each tube. Tubes were recapped and vortexed for 1 min and then set on a lab bench until layer separation. The upper organic phase was collected for Gas Chromatography (GC) analysis.

Microbial sample preparation: Approximately, 300 mg vacuum-freeze-dried microbial sample was ground to a powder followed by addition of 2 mL of benzene:petroleum ether (v/v = 1:1) to extract lipids. The extracted lipids in microbial samples were transesterified for 15 min in 2 mL of 0.5 sodium hydroxide-methanol solution (mol L^{-1}) (NaOH-CH_3OH). During this process, the tube was capped and placed in a water bath at 50°C for 15 min. Phase separation was achieved by addition of 1 mL deionized water and 2 mL hexane followed by vortexing for 1 min. Samples were placed on lab bench until phase separation took place. The upper phase was collected for GC analysis.

Culture medium sample preparation: Acidification of culture medium samples was achieved with 1 mL of hydrochloric acid (6 mol L^{-1}). Lipids were extracted from culture medium twice using chloroform/methanol solution. The extracted lipid was then transesterified using 2 mL of 0.5 mol L^{-1} sodium hydroxide-methanol (NaOH-CH_3OH) at in a 50°C water bath for 15 min. Afterwards, the upper organic phase was obtained for GC analysis by following the separation steps as the other sample described before.

FA standard preparation: Fatty acid standard was purchased from Sigma Chemical Co. (St. Louis, MO, USA) including Myristic Acid (MYA), Pentadecanoic Acid, Palmitic Acid (PtA), Palmitoleic Acid (PEA), heptadecanoic acid, Stearic Acid (StA), Oleic Acid (OLA), Linoleic Acid (LLA), Linolenic Acid (LNA), Erucic Acid and Arachidonic Acid (ADA). The transesterification reaction and other steps for the mixed standard were the same as for oil samples described before. The standard

was used to indentify the corresponding fatty acids in the samples measured in this experiment according to the chromatographic peak elution time. Identification of the peaks included fatty acids between C14:0 and C20:4.

GC analyses condition of fatty acid: Analyses were performed with a fused silica capillary column (DB-FFAP), 30 m × 0.25 mm Inner Diameter (ID) × 0.25 μm film thickness (J and W Scientific, Agilent Technologies), a split/splitless injector, an automatic sampler (Model AOC-17) and a flame ionization detection in a SHIMADZU GC-14B gas chromatograph (Shimadzu Corp., Kyoto, Japan). Initial temperature program was: 130°C with a 1 min hold; ramp: 4°C min^{-1} to 178°C, 1°C min^{-1} to 225°C and then 40°C min^{-1} to 245°C with a 13 min hold. Carrier gas was H_2 with a linear velocity of 60 cm sec^{-1}; a constant pressure of 102.4 kPa was used. Fatty acid analysis was performed by autoinjection of 1 μL of each sample at a 0 split ratio of 7.5:1. The FID temperature was 250°C with air and nitrogen make-up gas flow rates of 450 and 10 mL min^{-1}.

Relative ratio of fatty acid calculation: Peaks were identified by comparison to retention times for the standard. The relative ratio of individual fatty acids was calculated by the peak normalization method using the expression:

$$R_{ifa}(\%)=\left(\frac{A_{ifa}}{A_{tfa}}\right)\times 100\%$$

Where:

R_{ifa} = The relative ratio of individual fatty acid

A_{ifa} = The chromatographic area units of the individual fatty acids whose relative ratio was to be determined

A_{tfa} = The chromatographic area for the total fatty acids determined

Statistical analysis: Results were presented as mean±standard error. Statistical analysis was carried out by ANOVA test with posthoc multiple comparison test of Tukey using SPSS VE16.0 for windows (SPSS Inc., Chicago, IL). Probability values <0.05 were considered significant and probability values <0.01 were considered highly significant. Correlation analysis between variants was done using Correlate Bivarate in SPSS Version 16.0 for windows.

RESULTS AND DISCUSSION

Fatty acid profiles and iodine values of substrate oil: Results of fatty acids profiles and iodine values for 4 substrate oils used in the current experiment were shown in Table 2. From Table 2, it was observed that linoleic acid content in corn oil and soybean oil was highest ($p<0.05$) followed by oleic acid and palmitic acid. The most remarkable difference between them was in linolenic acid which in soybean oil was 9.1× higher than corn oil. Oleic acid was highest ($p<0.05$) in peanut oil followed by linoleic acid, palmitic acid and arachidonic acid. Additionally, arachidonic acid in peanut oil was 3-20× higher than other oils. Erucic acid was highest ($p<0.05$) in rapeseed oil followed by oleic acid, linoleic acid and linolenic acid. As shown by the iodine values, the unsaturated degree of oils determined in the current trial was quite different from each other depending on their fatty acid profile. The iodine values ranked in order from low to high was peanut oil (84.70), rapeseed oil (109.30), corn oil (125.50) and soybean oil (140.60).

Fatty acid profiles of culture medium: Stearic acid (average of 57.46%), palmitic acid (average of 16.50%) and oleic acid (average of 10.77%) in 24 h culture medium was comparatively higher regardless of treatments (Table 3). The results displayed on Table 3 showed that between

Table 2: Fatty acids profiles and iodine value of 4 substrate oils using in current experiment

Fatty acids (%)	A (Peanut oil)	B (Rapeseed oil)	C (Corn oil)	D (Soybean oil)	Mean	F-value	p-value
C14:0	0.82±0.06^{A}	0.32±0.03^{C}	0.19±0.02^{C}	0.60±0.02^{B}	0.48±0.08	70.76	0.00
C15:0	0.40±0.04^{B}	0.30±0.02^{B}	0.61±0.03^{A}	0.30±0.02^{B}	0.40±0.04	28.27	0.00
C16:0	13.43±0.38^{Aa}	4.78±0.20^{C}	11.67±0.43^{Ab}	9.23±0.43^{B}	9.78±0.99	100.12	0.00
C17:0	0.50±0.04^{C}	0.61±0.04^{BCc}	1.23±0.09^{Aa}	0.91±0.06^{ABb}	0.81±0.09	28.77	0.00
C18:0	2.20±0.11^{B}	4.01±0.33^{A}	1.80±0.11^{Bb}	2.66±0.12^{Ba}	2.67±0.26	25.01	0.00
SFA[1]	17.35±0.45^{A}	10.02±0.28^{C}	15.51±0.55^{AB}	13.70±0.44^{B}	14.14±0.84	50.45	0.00
C16:1	2.10±0.12^{A}	0.70±0.06^{B}	0.88±0.07^{B}	0.81±0.04^{B}	1.12±0.17	66.99	0.00
C18:1	46.23±1.64^{A}	18.00±0.74^{B}	20.40±0.85^{B}	20.60±0.99^{B}	26.31±3.51	144.36	0.00
C22:1	0.14±0.01^{B}	44.70±1.27^{A}	0.54±0.04^{B}	0.71±0.05^{B}	11.52±5.78	1202.56	0.00
MUFA[2]	48.47±1.55^{B}	63.40±0.61^{A}	21.82±0.89^{C}	22.13±1.04^{C}	38.95±5.38	361.19	0.00
C18:2	21.10±1.17^{B}	10.90±0.53^{C}	54.57±2.03^{Aa}	50.67±1.74^{Ab}	34.31±5.68	212.43	0.00
C18:3	0.73±0.05^{C}	9.11±0.30^{A}	0.70±0.04^{C}	6.40±0.32^{B}	4.24±1.10	357.36	0.00
C20:4	6.51±0.36^{A}	0.29±0.02^{C}	2.04±0.14^{B}	1.67±0.08^{B}	2.63±0.71	187.40	0.00
PUFA[3]	28.34±1.38^{Ba}	20.30±0.82^{Bb}	57.31±1.90^{A}	58.73±2.05^{A}	41.17±5.20	149.90	0.00
Iodine value	84.70	109.30	125.50	140.60			

$^{a-c}$Values with different small letter superscripts in the same row meant significantly different ($p<0.05$); $^{A-C}$Values with different capital superscripts in the same row meant very different ($p<0.01$); [1]SFA = Fatty acids without any double bond (14:0, 15:0, 16:0, 17:0 and 18:0). [2]MUFA = Fatty acids with a single double bond (16:1, 18:1 and 22:1). [3]PUFA = Fatty acids with 2 or more double bond (18:2, 18:3 and 20:4)

Table 3: Fatty acids profiles in 24 h culture medium from different oils

Fatty acids (%)	A (Peanut oil)	B (Rapeseed oil)	C (Corn oil)	D (Soybean oil)	Mean	F-value	p-value
C14:0	1.00±0.070	0.91±0.050	0.83±0.080	0.92±0.070	0.91±0.030	0.9800	0.45
C15:0	0.29±0.030	0.21±0.020	0.30±0.020	0.22±0.020	0.25±0.010	4.2200	0.05
C16:0	17.97±0.660^{a}	16.03±0.650^{ab}	17.03±0.550^{ab}	14.97±0.570^{b}	16.50±0.430	4.5300	0.04
C17:0	0.41±0.020^{B}	0.31±0.020^{B}	0.61±0.040^{A}	0.41±0.030^{B}	0.43±0.040	17.700	0.00
C18:0	60.07±1.200^{ABa}	55.03±1.630^{ABb}	61.60±1.230^{A}	53.13±1.030^{B}	57.46±1.190	9.6900	0.00
SFA[1]	79.72±0.720^{A}	72.49±0.980^{B}	80.37±0.750^{A}	69.64±0.770^{B}	75.56±1.430	43.220	0.00
C16:1	1.20±0.070^{Aa}	1.00±0.070^{AB}	0.80±0.040^{B}	0.91±0.040^{ABb}	0.98±0.050	8.6100	0.01
C18:1	10.03±0.270^{B}	7.02±0.320^{Cb}	9.02±0.400^{BCa}	17.00±0.570^{A}	10.77±1.150	114.03	0.00
C22:1	0.10±0.010^{B}	9.64±0.370^{A}	0.40±0.020^{B}	0.31±0.030^{B}	2.61±1.230	635.80	0.00
MUFA[2]	11.33±0.340^{B}	17.66±0.660^{A}	10.23±0.350^{B}	18.22±0.570^{A}	14.36±1.110	69.030	0.00
C18:2	3.01±0.230^{B}	2.99±0.070^{B}	4.03±0.240^{ABb}	4.93±0.200^{Aa}	3.74±0.260	22.390	0.00
C18:3	0.81±0.040^{C}	1.54±0.080^{B}	0.69±0.050^{C}	2.01±0.120^{A}	1.26±0.170	61.020	0.00
C20:4	0.71±0.050^{A}	0.10±0.090^{B}	0.30±0.020^{B}	0.20±0.020^{B}	0.33±0.070	81.290	0.00
PUFA[3]	4.53±0.220^{B}	4.64±0.150^{B}	5.02±0.310^{B}	7.14±0.340^{A}	5.33±0.340	21.180	0.00

Table 4: Fatty acids profiles of rumen microorganisms with different oils

Fatty acids (%)	A (Peanut oil)	B (Rapeseed oil)	C (Corn oil)	D (Soybean oil)	Mean	F-value	p-value
C14:0	1.01±0.073	1.21±0.040	1.05±0.090	1.00±0.080	1.07±0.040	1.810	0.220
C15:0	0.09±0.010^{b}	0.10±0.010^{b}	0.15±0.020^{a}	0.10±0.010^{b}	0.11±0.010	6.230	0.020
C16:0	28.22±0.480	26.47±0.230	27.86±0.190	26.91±0.830	27.37±0.300	2.628	0.122
C17:0	0.41±0.030^{A}	0.41±0.030^{A}	0.30±0.030^{AB}	0.21±0.030^{B}	0.33±0.030	10.56	0.000
C18:0	35.18±0.580	36.03±0.440	37.08±0.190	36.07±0.430	36.09±0.270	3.260	0.080
SFA[1]	64.91±0.570^{ab}	64.22±0.630^{b}	66.44±0.110^{a}	64.29±0.440^{ab}	64.97±0.340	4.550	0.040
C16:1	1.19±0.060	1.21±0.050	1.21±0.080	1.20±0.070	1.20±0.030	0.020	1.000
C18:1	17.84±0.780^{ab}	18.07±1.070^{ab}	16.31±0.500^{b}	19.98±0.670^{a}	18.05±0.520	3.690	0.060
C22:1	0.16±0.010^{B}	0.30±0.020^{A}	0.15±0.010^{B}	0.15±0.010^{B}	0.19±0.020	24.67	0.000
MUFA[2]	19.19±0.760	19.58±1.020	17.66±0.510	21.33±0.620	19.44±0.510	4.010	0.050
C18:2	9.62±0.360	8.53±0.760	9.70±0.430	9.09±0.770	9.24±0.300	0.790	0.530
C18:3	0.81±0.040	0.79±0.050	0.72±0.050	0.84±0.060	0.79±0.020	1.060	0.420
C20:4	2.40±0.153	2.00±0.150	2.50±0.150	2.30±0.110	2.30±0.080	2.300	0.150
PUFA[3]	11.33±0.880	12.92±0.540	12.23±0.720	12.33±0.350	11.33±0.880	1.140	0.390

$^{a-c}$Values with different small letter superscripts in the same row meant significantly different ($p<0.05$); $^{A-C}$Values with different capital superscripts in the same row meant very different ($p<0.01$); [1]SFA = Fatty acids without any double bond (14:0, 15:0, 16:0, 17:0 and 18:0). [2]MUFA = Fatty acids with a single double bond (16:1, 18:1 and 22:1). [3]PUFA = Fatty acids with 2 or more double bond (18:2, 18:3 and 20:4)

treatments significant differences were found in 9 of the fatty acids identified ($p<0.05$) except for myristic acid ($p = 0.45$) and pentadecanoic acid ($p = 0.05$).

For instance, palmitic acid in peanut oil was significantly higher than soybean oil ($p = 0.04$) and arachidonic acid in peanut oil was higher than the other 3 groups ($p = 0.00$). Corn oil led to the highest heptadecanoic acid concentration ($p = 0.00$) and rapeseed oil led to the highest erucic acid concentration ($p = 0.00$) in culture medium among treatments. Additionally, linoleic acid was significantly higher due to soybean oil than corn oil ($p<0.05$) or peanut oil and rapeseed oil ($p<0.01$).

Fatty acid profiles of rumen microorganisms: The result of fatty acid composition of mixed rumen microorganisms in culture were shown in Table 4. Overall, results showed that regardless of treatments stearic acid (average of 36.09%), palmitic acid (average of 27.37%) and oleic acid (average of 18.05%) were the most abundant FA in rumen microorganisms.

The results showed that between treatments significant differences were found only in pentadecanoic acid, heptadecanoic acid, oleic acid and erucic acid. Pentadecanoic acid due to corn oil was higher than other oils ($p = 0.02$), heptadecanoic acid due to peanut oil or rapeseed oil was higher than soybean oil ($p = 0.00$), oleic acid due to soybean oil was significantly higher than corn oil ($p = 0.04$) and erucic acid due to rapeseed oil was significantly higher than other oils ($p = 0.00$).

Correlation analysis of fatty acids: The results described above showed that the fatty acid profiles of culture medium or microbes could be manipulated by the substrate oil and so the correlation analyses of fatty acids between substrate oils and culture medium or microorganisms were conducted to further elucidate. The analysis results shown in Table 5 showed that among 11 kinds of fatty acids determined in total, significant relationships existed in 8 kinds of fatty acids between 24 h culture medium and substrate oils (2-tailed <0.05 or 2-tailed<0.01); it was however that significant relationships only existed in 4 kinds of fatty acids between culture organisms and substrate oils which respectively were pentadecanoic acid (2-tailed = 0.001), palmitic acid (2-tailed = 0.029), heptadecanoic acid (2-tailed = 0.041) and erucic acid (2-tailed = 0.000).

Table 5: Correlation analysis of fatty acids between oils and culture medium or microorganisms

Correlation analysis	Item	C14:0	C15:0	C16:0	C17:0	C18:0	C16:1	C18:1	C22:1	C18:2	C18:3	C20:4
Oils and culture medium	Pearson correlation	0.462	0.583*	0.476	0.818**	-0.607*	0.669**	-0.057	0.994**	0.830**	0.818**	0.984**
	Sig. (2-tailed)	0.130	0.046	0.117	0.001	0.036	0.017	0.862	0.000	0.001	0.001	0.000
	N	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000
Oils and microorganisms	Pearson correlation	-0.346	0.829**	0.626*	-0.597*	-0.096	-0.121	-0.070	0.934**	0.216	0.261	0.433
	Sig. (2-tailed)	0.270	0.001	0.029	0.041	0.766	0.708	0.828	0.000	0.499	0.413	0.160
	N	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000	12.000

*Correlation was significant at the 0.05 level (2-tailed); **Correlation was significant at the 0.01 level (2-tailed)

Effects on fatty acid profiles in culture medium: Fat supplements are included in the diet of ruminants to increase energy density, improve nutrient utilization, enhance milk and meat yields and affect fatty acid composition (Bauman *et al*., 2003). It is known that dietary lipids undergo two important transformations in ruminant rumen. Rumen metabolism of dietary fat is initiated by microbial lipolysis and subsequent Biohydrogenation (BH) of free PUFA (Fellner *et al*., 1995; Harfoot and Hazlewood, 1997).

The modification of the microbial population and fermentation due to the intoxication of free fatty acids lipolyzed by microbes exist in the rumen (Dohme *et al*., 2000) and the extent of this effect are related to the added amount of lipids but also to their degree of unsaturation (Jalc *et al*., 2002). In current study, saturated fatty acids were comparatively higher in 24 h culture medium with stearic acid and palmitic acid holding high levels. In detail, palmitic acid ratio in culture was 1.34, 3.35, 1.46 and 1.62 times as high as those in corresponding substrate oils as for stearic acid, the ratio was 27.30, 13.72, 34.22 and 19.97 times as high as that in corresponding substrate oils. Quite the reverse, oleic acid ratio was high in substrate oils which was 4.55, 2.56, 2.27 and 1.20 times as high as that in 24 h culture medium correspondingly.

Unsaturated fatty acids might be converted to saturated fatty acids by BH (Kemp and Lander, 1984; Loor *et al*., 2003) this then was proved by current results described above showing the saturated fatty acids increased after a 24 h culture. And moreover, the changes of different substrate oils used in current experiment differed from each other. For instance, oleic acid content of soybean oil was similar to that of corn oil and lower than that of peanut oil however, oleic acid content in 24 h culture medium due to soybean oil was significant higher than that in 24 h culture medium from corn oil or peanut oil.

The most likely reason might be that both linoleic acid and linolenic acid content were much higher in soybean oil this then might inhibit BH completely to stearic acid by decreasing the activity of B type bacteria during BH process and consequently, caused the accumulation of trans-oleic acid produced by A type bacteria (Griinari and Bauman, 1999; Yuzhi *et al*., 2005; Hou *et al*., 2008). Series of isomeric fatty acids (cis/trans) such as trans-oleic acid should be further separated in order to elucidate the fatty acid metabolism in rumen. Meanwhile in this study, erucic acid was higher in 24 h culture medium due to rapeseed oil than the other groups; linoleic acid was high in 24 h culture medium due to corn oil or soybean oil, compared to peanut oil or rapeseed oil. And as for the 24 h culture medium due to peanut oil, it showed a high arachidonic acid ratio, compared with the other 3 groups. Those results indicated that fatty acid compositions of 24 h culture medium was association with the fatty acid compositions of oil used in substrate this then was further supported by the results of the correlation analysis which the significant relationships existed in 8 kinds of fatty acids between 24 h culture medium and substrate oil. And that meant, fatty acids composition of culture was modified by substrate oil in some degree.

Effects on fatty acids profiles in rumen microorganisms: Rumen bacteria dry matter has a fat content of between 10 and 15% and liquid-associated bacteria are lower than solid-adherent bacteria in fat content (Bauchart *et al*., 1990). There are two pathways for the formation of bacterial fat, the one is de novo synthesis and the other is absorbed from dietary long-chain fatty acid. The contributing ratio of these 2 pathways depends on the dietary fat contents, bacterial species, etc. The main productions of de novo synthesis pathway are stearic acid and palmitic acid in a ratio of 2:1 of stearic acid to palmitic acid (Fellner *et al*., 1995). Monounsaturated fatty acid can be produced via anaerobic pathway by fatty acid desaturases catalyzing with palmitoleic acid and oleic acid as the end production (Fellner *et al*., 1995). Rumen bacteria can not only synthesize fat itself but convert unsaturated fatty acid through BH; it is therefore speculated that fatty acid composition of rumen bacteria might not be manipulated by substrate oils.

In present study, stearic acid, palmitic acid and oleic acid was abundant in rumen organisms regardless of treatments. This result coincided with the recent report of rumen microbes by Sultana *et al*. (2011) showing the proportions of C16:0 and C18:0 in total fatty acids in bacterial cells were all >20% (20.7 and 37.4%, respectively)

and also agreed with the earlier research described by Or-Rashid *et al.* (2007). Whittaker *et al.* (2005) ever suggested that these fatty acid intensity profiles were unique for species and that they could be used as a fingerprint for the organisms. The results of current research also demonstrated that the fatty acids compositions of rumen microbes unchanged with substrates compared with the culture medium which might be looked as the particular feature of rumen microbes. The relationship analysis subsequently confirmed that significant relationships only existed in 4 kinds of fatty acids between microbes and substrate oils which respectively were pentadecanoic acid, palmitic acid, heptadecanoic acid and erucic acid.

The possible reason might be that as the researchers knowledge, pentadecanoic acid, heptadecanoic acid and erucic acid were low of their ratios in ruminal bacterial fatty acid composition (Or-Rashid *et al.*, 2007) or generally not even being examined (Vasta *et al.*, 2010) that meant these were not the common fatty acids in rumen bacteria. And so, their ratios might be higher or lower in bacterial cell depending on the corresponding ratios in environment. Differently, palmitic acid existed in rumen bacteria commonly with a high ratio, the reason why it also varied with substrate oil could not yet be explained in this research. To clarify this reason would be interesting by further researches.

CONCLUSION

The results of this study demonstrated that stearic acid, palmitic acid and oleic acid in 24 h culture medium were comparatively higher and moreover, the fatty acids profile of 24 h culture medium was modified by substrate oil in some degree. Additionally, stearic acid palmitic acid and oleic acid ratios were comparatively higher in rumen microbes; it was however that the fatty acids compositions in rumen microbes might be unchangeable, compared with that in culture medium.

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