Effect of Concentrate Level on the Formation of Conjugated Linoleic Acid and *Trans*-octadecenoic Acid by Ruminal Bacteria when Incubated with Oilseeds *In Vitro**

J. H. Wang, M. K. Song*, Y. S. Son[1] and M. B. Chang[2]
Department of Animal Science, Chungbuk National University, Chungbuk 361-763, Korea

ABSTRACT : An *in vitro* study was conducted to examine the effect of addition level of concentrate on fermentation characteristics and long-chain unsaturated fatty acids composition, especially conjugated linoleic acid (CLA) and *trans*-octadecenoic acid (t-FA) by mixed ruminal bacteria when incubated with linseed or rapeseed. Four levels (0.83, 1.25, 1.67 and 2.08%, w/v) of concentrate and ground oilseeds (linseed or rapeseed; 0.83%, w/v) were added to mixed solution of strained rumen fluid with artificial saliva (1:1, v/v) in the glass jar with a glass lid equipped with stirrer, and was incubated anaerobically for 24 h at 39°C. Addition level of concentrate slightly reflect on pH and ammonia concentration of the culture solution at the various incubation times when incubated with both linseed and rapeseed. Total VFA concentration slightly increased with incubation times and concentrate levels for incubations with oilseeds. While CLA composition had a clearly increasing trend with incubation time when incubated with linseed, percent CLA was relatively stable when incubated with rapeseed. Percent CLA, however, had a clearly decreasing trend with concentrate level throughout incubation times with significances at 3 h incubations when incubated with linseed ($p<0.038$) and rapeseed ($p<0.0009$). The differences in compositions of t-FA were relatively small among concentrate levels for both incubations with linseed and rapeseed. The ratios of t-FA to CLA were lower for linseed with increased proportion of CLA than for rapeseed. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 5 : 687-694)***

Key Words : Concentrate Level, Oilseed, Bio-Hydrogenation, CLA, Octadecenoic Acid, *In Vitro*, Mixed Ruminal Bacteria

INTRODUCTION

The bio-hydrogenation activity of ruminal microbes is very high (Wu et al., 1991; Huang et al., 1999; Wang and Song, 2001) although fatty acid compositions in beef and milk have been affected, to some extent, by feeding vegetable oils or oilseeds (Kennelly, 1996; Chouinard et al., 1998a) Conjugated linoleic acid (CLA) and *trans*-$C_{18:1}$ fatty acid (t-FA) are formed as a result of incomplete bio-hydrogenation of unsaturated fatty acids in the rumen (Wu et al., 1991). Limited number of bacterial spp were reported to be able to produce CLA from free linoleic acid ($C_{18:2}$) *in vitro* (Jiang et al., 1998).

While the CLA exhibits promising beneficial health effects (NRC, 1996) the t-FA has the negative effects on animal performance (Kennelly, 1996) and human health (Erasmus, 1993). The contents of CLA and t-FAs, however, tend to be positively correlated in rumen contents (Bessa et al., 2000). Bessa et al. (2000) also indicated that nutritional strategies for the enrichment of ruminant products with CLA could be achieved by increasing the supply of $C_{18:2}$ in reticulo-rumen, although this would also result in an increase in t-FA. Kim et al. (2000) also reported that growing cultures did not produce significant amounts of *cis*-9, *trans*-11 CLA until the $C_{18:2}$ concentration was high enough to inhibit bio-hydrogenation.

Thus, it might be important to manipulate the ruminal bio-hydrogenation in order to increase CLA output with a low t-FA/CLA ratio. Because the extent of bio-hydrogenation by ruminal bacteria could be affected by concentrate to roughage ratio (Leat, 1977) and ruminal pH (Song and Choi, 1998; Wang and Song, 1999) demand for the dietary manipulation relating to the concentrate levels and lipid sources has been increased in the examination of the transformation of C_{18}-unsaturated fatty acids by rumen microbes.

Therefore, the present *in vitro* study was conducted to examine the effect of addition level of concentrate on fermentation characteristics and C_{18}-long chain fatty acids composition, especially CLA and t-FA by ruminal bacteria when used two lipid sources consisting of different fatty acids.

MATERIALS AND METHODS

Preparation of rumen fluid

Rumen contents were collected at 3 h after morning feeding (06:00) from the ruminally cannulated Holstein cow fed 5 kg of corn silage (60%) and concentrate (40%) on a

* Address reprint request to M. K. Song. Tel: +82-43-261-2545, E-mail: mksong@cbucc.chungbuk.ac.kr
[1] Department of Animal Science, Korea University, Korea.
[2] Department of Animal Science and Technology, College of Industrial Sciences, Chung-ang University, Korea.
Received November 14, 2001; Accepted January 22, 2002

dry matter (DM) basis twice daily. The rumen contents were brought to the laboratory and were blended in a Waring blender (Fisher 14-509-1) for 20 seconds at high speed to detach the bacteria from the feed particles, and were strained through 12 layers of cheesecloth to remove the feed particles and large protozoa. CO_2 was flushed into the strained rumen fluid.

Preparation and incubation of culture

Strained rumen fluid was mixed with McDougall's artificial saliva (1948) at the ratio of 1:1 under flushing of CO_2. Four levels (0.83, 1.25, 1.67 and 2.08%, w/v) of concentrates and ground (1 mm) linseed (*Linum. usitatissimus*) or rapeseed (*Brassica. napus*) at the level of 0.83% (w/v) were added to 1,200 ml mixed solution in the glass culture jar, and CO_2 was flushed into the culture solution for 3 minutes. The culture jar was covered with a glass lid equipped with stirrer and was placed into a water-bath (39°C). Culture solution was again flushed with CO_2 through glass tube connected to the jars for the infusion purpose for 3 min., and was incubated up to 24 h. Stirring speed during incubation was adjusted to 120 times/min. The incubation of culture solution was done three times under similar condition.

Enumeration of total viable bacteria

The number of viable bacteria in culture solution was determined at 12 h and 24 h incubation by the anaerobic culture techniques of Hungate (1966). One ml of culture solution from each treatment was taken and diluted to 10^5-10^7 using the Bryant's diluting solution (1961). Then, 1ml diluted solution was inoculated into the roll tube containing non-selective artificial medium (Scott and Dehority, 1965). The number of colony was counted after the roll tubes were incubated at 39°C for 5 days.

Sampling and analysis

pH of culture solution was measured at the incubation times of 3, 6, 12 and 24 h, and 5 ml culture solution was collected for ammonia and volatile fatty acid (VFA) analysis. All samples collected were kept frozen at -20°C until analyzed. Ammonia concentration was determined by the method of Fawcett and Scott (1960) using the spectrophotometer (DU-650). Four ml culture solution was mixed with 1 ml 25% phosphoric acid and 0.5 ml pivalic acid solution (2%, w/v) as an internal standard. The mixed solution was centrifuged at 15,000×g for 15 min., and the supernatant was used to determine the concentration and composition of VFA using gas chromatograph (GC, HP 5890Ⅱ, Hewlett Packard Co.). Two hundred ml incubation solution was also collected at the incubation times of 3, 6, 12 and 24 h, and freeze dried and lipids were extracted using Folch' s solution (Folch et al., 1957). Methylation of the lipids followed the method of Lepage and Roy (1986) prior to injecting into the GC, using a fused silica capillary column (100 m×0.25 mm, i.d.×0.20 μm thickness, Supelco, SPTM-2560; USA).

Statistical analysis

The results obtained were subjected to least squares analysis of variance according to the general linear models procedure of SAS (1985) and significances were compared by S-N-K Test (Steel and Torrie, 1980).

RESULTS

Compositions of C_{18}-fatty acids in oilseeds and concentration were presented in table 1. Composition of linolenic acid ($C_{18:3}$) in both linseed and rapeseed was highest as 59.2% and 34.0%, respectively, and followed by oleic acid ($C_{18:1}$) and $C_{18:2}$. But $C_{18:2}$ composition was highest in concentrate. Total lipids in culture solution were shown in table 2. Concentrate additions increased the amount of lipids at the ranges of 0.64 to 1.61 g in culture solutions containing both linseed and rapeseed.

Addition level of concentrate did not clearly reflect on pH of the culture solution at the various incubation times but higher concentrate levels had the trends of low pH than lower levels of concentrate when incubated with both linseed and rapeseed (figure 1). No differences were observed in ammonia concentration among concentrate levels although it tended to decrease with the level of concentrate for incubations with both oilseeds (figure 2). There were increasing trends in total VFA concentration with incubation time and concentrate level for both incubations with linseed (table 3) and rapeseed (table 4),

Table 1. Lipid contents and C_{18}-fatty acid composition of oilseeds and concentrate

Feed Components	Lipid (%, DM)	Composition of C_{18}-Fatty acid (%)			
		Stearic acid ($C_{18:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)	Linolenic acid ($C_{18:3}$)
Linseed	34.04	2.75	16.9	13.9	59.2
Rapeseed	43.83	3.22	31.1	22.3	34.0
Concentrate	6.42	4.77	23.5	43.1	4.5

Table 2. Lipids in culture solution (g/1,200 mL)

Treatments (Oilseeds)	Addition levels of concentrate (%, w/v)*			
	0.83	1.25	1.67	2.08
Linseed + Con.	4.03	4.36	4.68	5.00
Rapeseed + Con.	5.01	5.33	5.65	5.98

* Lipid content of concentrate was 6.42% on a DM basis.

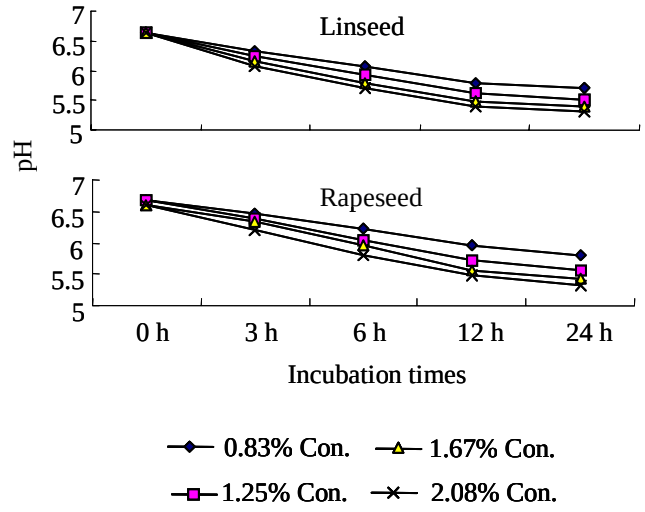

Figure 1. pH of culture solution.

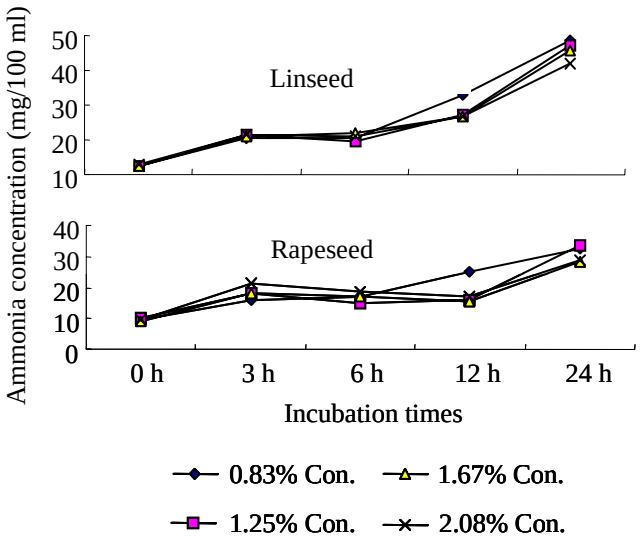

Figure 2. Ammonia concentration in culture solution

and higher levels of concentrate addition significantly increased total VFA concentration in culture solution incubated with linseed at 6 h ($p<0.022$, table 3) and with rapeseed at 24 h ($p<0.030$, table 4). As expected, molar percent of acetate (C_2) had a decreasing trend while that of propionate (C_3) tended to increase with incubation times and concentrate level for both incubations with linseed (table 3) and rapeseed (table 4). Although no clear differences among concentrate levels were observed in molar percent of C_2 for both incubations with oilseeds, that of C_3 was increased by the highest addition level of concentrate 12 h ($p<0.029$) and 24 h ($p<0.030$) when incubated with linseed. Bacterial number in culture solution had an increasing trend with concentrate level at incubations of 24 h (linseed, table 3) and 12 h (rapeseed, table 4).

Decreasing trends in the compositions of oleic acid ($C_{18:1}$), $C_{18:2}$ and $C_{18:3}$ but increasing trends of stearic acid ($C_{18:0}$) and t-FA compositions were found from culture contents with incubation time when incubated with both linseed (table 5) and rapeseed (table 6) in the all concentrate levels. But while CLA composition had a clearly increasing trend with incubation time when incubated with linseed (table 5) percent CLA was relatively stable when incubated with rapeseed (table 6). When incubated with linseed, the concentrate level did not affect compositions of $C_{18:0}$ and $C_{18:1}$, but that of $C_{18:2}$ tended to increase with concentrate level for all incubation times while $C_{18:3}$ tended to decrease up to 6 h, thereafter increased with concentrate level (table 5). But when incubated with rapeseed, both $C_{18:2}$ and $C_{18:3}$ tended to increase with concentrate level from 6h incubation (table 6). The differences in compositions of t-FA were relatively small among concentrate levels up to 12 h incubation but decreasing trend was observed with concentrate level at 24 h when incubated with linseed (table 5). Decreasing trends also were found in t-FA composition as incubation proceeded when incubated with rapeseed (table 6). Percent CLA had a clearly decreasing trend with concentrate level throughout incubation times with significances at 3 h incubations when incubated with linseed ($p<0.038$, table 5) and rapeseed ($p<0.0009$, table 6). The t-FA/CLA ratios between oilseeds differed with incubation times and with concentrate levels (tables 5 and 6). The ratio was lower for linseed with increased proportion of CLA than for rapeseed although the ratio tended to increase with the concentrate level.

DISCUSSION

The degradations of concentrate and oilseeds, and the bio-hydrogenation of C_{18}-unsaturated fatty acids are mostly considered to be the results of bacterial activity in the present study since protozoa were seldom found from the strained rumen fluid under the microscope due to the straining of rumen contents through 12 layers of cheesecloth. Based on the pH (figure 1), ammonia

Table 3. Concentration, molar proportion of VFA and number of viable bacteria in culture solution when incubated with linseed

Items	Concentrate[1), 2)]				SEM[3)]	Pr>F[4)]
	0.83	1.25	1.67	2.08		
			3 h			
Total VFA (mmoles/100 ml)	62.05	63.28	69.99	69.34	3.922	0.503
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	48.62	47.87	47.34	46.41	1.155	0.628
Propionate (C_3)	26.85	28.05	28.96	29.96	0.816	0.189
C_2/C_3	1.81	1.71	1.64	1.55	0.088	0.325
			6 h			
Total VFA (mmoles/100 ml)	66.71^{b}	74.03^{ab}	81.14^{a}	83.60^{a}	2.314	0.022
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	47.85	46.6	45.60	44.37	1.409	0.443
Propionate (C_3)	26.79	28.02	29.09	30.10	0.683	0.096
C_2/C_3	1.79	1.67	1.57	1.48	0.088	0.221
			12 h			
Total VFA (mmoles/100 ml)	85.1	92.97	97.08	102.67	6.140	0.354
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	46.79	45.74	44.44	42.92	1.070	0.203
Propionate (C_3)	26.58^{b}	27.57^{ab}	28.50^{ab}	29.35^{a}	0.393	0.029
C_2/C_3	1.76	1.66	1.56	1.46	0.059	0.087
Bacteria ($\times 10^7$)	56.75	33.65	46.75	41.85	21.200	0.886
			24 h			
Total VFA (mmoles/100 ml)	99.69	108.13	113.69	121.06	5.911	0.216
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	45.17	43.80	41.81	40.13	1.169	0.124
Propionate (C_3)	26.25^{b}	27.18^{ab}	27.51^{ab}	28.58^{a}	0.323	0.031
C_2/C_3	1.72^{a}	1.61^{ab}	1.52^{ab}	1.40^{b}	0.053	0.054
Bacteria ($\times 10^7$)	12.65	22.25	44.15	48.90	13.440	0.309

[1)] Addition levels of concentrate (%, w/v) into 1,200 ml culture solution.
[2)] Means in the same row with different superscripts differ.
[3)] Standard error of the mean.
[4)] Probability levels.

concentration (figure 2) and VFA production (tables 3 and 4), fermentation characteristics were reflected by the concentrate levels in both incubations with oilseeds, but VFA concentrations with incubation times were slightly higher for linseed addition than for rapeseed, indicating greater fermentation of culture solution containing linseed. *Trans–11* $C_{18:1}$ was the major isomer of oleic acid, and *trans-7* and *trans-9* isomers were detected a little in the present study, thus they were combined with the *trans-11* isomer as a total t-FA of $C_{18:1}$. Only *cis*-9, *trans*-11 isomer was detected in the present study and it has been the major type of CLA (Parodi, 1997; Sehat et al., 1998).

The changes in t-FA and CLA production with incubation times indicated that the initial step occurred rapidly, but higher concentrate level reduced the extent of bio-hydrogenation of $C_{18:2}$ and $C_{18:3}$ and isomerization of $C_{18:2}$ in culture solution containing oilseeds, resulting in reduced proportions of t-FA and CLA (tables 5 and 6). The reduced proportions of t-FA and CLA might be caused by the lowered pH (figure 1) as addition level of concentrate increased. The possible pH effect on CLA production was released by Hughes et al. (1982) as *cis*-9, *trans*-11 octadecadienoate reductase activity of *Butirivibrio fibrisolvens* was stimulated at the higher pH and had a maximum activity for pH between 7.2 and 8.2. Similar result was found from the *in vivo* study of Kelly and Bauman (1996) where the CLA levels in milk were halved when the forage to concentrate ratio was changed from 50:50 to 20:80. Griinari et al. (1996) also reported that relatively increased forage feeding to concentrate increased milk fat concentrations of CLA.

The bio-hydrogenation pathway of $C_{18:2}$ has involved an

Table 4. Concentration, molar proportion of VFA and number of viable bacteria in culture solution when incubated with rapeseed

Items	Concentrate[1), 2)]				SEM[3)]	Pr>F[4)]
	0.83	1.25	1.67	2.08		
			3 h			
Total VFA (mmoles/100 ml)	51.77	57.44	63.02	64.37	6.759	0.586
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	50.87	50.01	49.52	48.35	1.866	0.813
Propionate (C_3)	26.11	27.46	28.41	29.72	0.923	0.178
C_2/C_3	1.95	0.83	1.75	1.63	0.126	0.432
			6 h			
Total VFA (mmoles/100 ml)	63.48	70.06	73.95	81.14	7.415	0.482
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	49.81	48.93	47.98	46.76	2.011	0.746
Propionate (C_3)	26.38	27.46	28.53	29.93	1.055	0.247
C_2/C_3	1.88	1.79	1.69	1.57	0.137	0.466
			12 h			
Total VFA (mmoles/100 ml)	74.84	87.34	90.44	97.44	6.323	0.226
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	48.55	46.62	46.43	44.39	1.692	0.475
Propionate (C_3)	26.56	27.95	27.84	29.48	0.963	0.335
C_2/C_3	1.83	1.67	1.67	1.51	0.116	0.398
Bacteria ($\times 10^7$)	33.15	42.75	48.75	51.40	14.510	0.817
			24 h			
Total VFA (mmoles/100 ml)	87.16^{b}	94.30^{ab}	108.39^{ab}	115.37^{a}	4.276	0.023
Molar proportion (mmoles/100 mmoles)						
Acetate (C_2)	47.62	45.95	44.74	42.46	1.785	0.347
Propionate (C_3)	25.79	26.81	27.59	28.55	0.808	0.241
C_2/C_3	1.85	1.72	1.62	1.49	0.116	0.297
Bacteria ($\times 10^7$)	12.55	13.55	14.80	16.80	6.340	0.965

[1)] Addition levels of concentrate (%, w/v) into 1,200 ml culture solution.

[2)] Means in the same row with different superscripts differ.

[3)] Standard error of the mean.

[4)] Probability levels.

initial isomerization step, resulting in the formation of *cis*-9, *trans*-11 CLA, which undergoes sequential reduction steps yielding *trans*-11-octadecenoic acid and then $C_{18:0}$ (Harfoot and Hazlewood, 1988). This sequential reduction steps of $C_{18:3}$ bio-hydrogenation pathway did not consider CLA as a intermediate but produce *trans-11* $C_{18:1}$ as one of the final products. Chouinard et al. (1998b) and Kelly et al. (1998) also indicated that CLA was mostly derived from the dietary $C_{18:2}$. The results of the present study, however, revealed greater proportions of CLA from linseed incubation (table 5) than from rapeseed (table 6). Linseed had a higher $C_{18:3}$ proportion than rapeseed (table 1). This indicates that an alternative pathway may exist in the bio-hydrogenation of $C_{18:3}$ to produce the CLA. Bessa et al. (2000) also postulated the possibility that alternative pathways may exist in the production of CLA from $C_{18:3}$ due to the extreme microbial diversity in the reticulo-rumen.

Both CLA and t-FA are all derived from rumen bio-hydrogenation of C_{18}- poly USFA, and the contents of CLA and t-FA are positively correlated in rumen contents, fat depots and milk (Solomon et al., 2000). But due to the impacts of both bio-hydrogenation intermediates on the human health, it may be important to manipulate rumen bio-hydrogenation toward the CLA increase with a low t-FA/CLA ratio. The t-FA/CLA ratios between oilseeds differed with incubation times and with concentrate levels (tables 5 and 6). The ratio was lower for linseed with increased proportion of CLA than for rapeseed although the ratio tended to increase with the concentrate level. This trend might be related to the rate of fermentation and in turn,

Table 5. Composition (%) of C_{18}-fatty acids in culture solution when incubated with linseed

Fatty acids	Concentrate[1), 2)]				SEM[3)]	Pr>F[4)]
	0.83	1.25	1.67	2.08		
			3 h			
$C_{18:0}$	26.13	26.99	26.58	26.70	1.369	0.974
$C_{18:1}$	14.81	14.76	14.6	14.87	0.590	0.994
t-$C_{18:1}$[5)]	5.99	6.88	6.48	6.77	0.850	0.913
CLA[6)]	1.13[a]	0.96[ab]	0.74[b]	0.81[b]	0.062	0.038
t-FA/CLA [7)]	5.31	6.97	8.84	8.30	0.758	0.097
$C_{18:2}$	8.99	9.44	10.58	10.68	0.439	0.122
$C_{18:3}$	26.09	24.76	24.41	23.48	2.331	0.883
			6 h			
$C_{18:0}$	28.09	27.23	28.96	28.56	3.434	0.985
$C_{18:1}$	14.21	15.65	15.00	15.49	0.684	0.815
t-$C_{18:1}$	6.55	6.05	8.07	6.63	0.464	0.128
CLA	1.44	1.31	1.38	1.17	0.101	0.391
t-FA/CLA	4.59	4.61	5.90	5.75	0.649	0.417
$C_{18:2}$	8.21	9.20	9.00	9.78	0.787	0.611
$C_{18:3}$	25.14	24.51	20.87	21.94	2.415	0.594
			12 h			
$C_{18:0}$	38.23	38.64	35.56	37.32	6.018	0.982
$C_{18:1}$	13.48	13.07	13.68	11.72	1.652	0.834
t-$C_{18:1}$	10.25	9.27	9.77	10.75	1.732	0.934
CLA	3.63	3.85	1.89	1.33	0.671	0.129
t-FA/CLA	2.81	2.76	5.17	8.32	1.234	0.089
$C_{18:2}$	4.79	5.73	6.60	6.36	0.999	0.621
$C_{18:3}$	12.13	14.24	15.63	15.73	2.615	0.754
			24 h			
$C_{18:0}$	39.22	39.54	36.71	35.46	1.864	0.227
$C_{18:1}$	9.02	12.48	13.53	11.15	2.208	0.091
t-$C_{18:1}$	17.35	15.01	15.51	12.31	3.893	0.834
CLA[6]	5.02	3.93	3.86	2.35	1.664	0.739
t-FA/CLA	3.46	3.81	4.03	6.82	1.863	0.659
$C_{18:2}$	3.55	3.44	3.98	5.78	0.734	0.230
$C_{18:3}$	7.70	7.49	8.74	14.17	2.061	0.216

[1)] Addition levels of concentrate (%, w/v) into 1,200 ml culture solution.
[2)] Means in the same row with different superscripts differ.
[3)] Standard error of the mean.
[4)] Probability levels.
[5)] Total trans-$C_{18:1}$ isomers.
[6)] Conjugated linoleic acid (*cis*-9, *trans*-11 isomer of linoleic acid).
[7)] The ratio of total trans-$C_{18:1}$ isomers to Conjugated linoleic acid.

fatty acid composition between oilseeds.

CONCLUSION

The fermentation characteristics and the extent of bio-hydrogenation of C_{18}-PUIFA, especially in the production of t-FA and CLA were influenced, to some extend, by both oilseeds and concentrate level. Greater CLA production from linseed which contains more $C_{18:3}$ than rapeseed may indicate that CLA could also be derived from the $C_{18:3}$.

Table 6. Composition (%) of C_{18}-fatty acids in culture solution when incubated with rapeseed

Fatty acids	Concentrate[1) 2)]				SEM[3)]	Pr>F[4)]
	0.83	1.25	1.67	2.08		
			3 h			
$C_{18:0}$	27.91	27.69	30.56	30.47	2.654	0.793
$C_{18:1}$	21.29	20.78	19.35	17.48	2.675	0.757
t-$C_{18:1}$[5)]	3.67	3.69	5.02	6.34	0.582	0.081
CLA[6)]	0.81[a]	0.61[c]	0.71[b]	0.70[b]	0.011	0.0009
t-FA/CLA[7)]	5.99	4.54	7.09	9.01	0.859	0.082
$C_{18:2}$	15.65	16.09	14.78	14.30	1.161	0.707
$C_{18:3}$	13.30	12.77	10.63	12.50	2.325	0.857
			6 h			
$C_{18:0}$	32.45	32.57	30.41	26.03	2.045	0.226
$C_{18:1}$	21.06	19.05	19.55	20.78	1.965	0.864
t-$C_{18:1}$	7.11	7.58	7.42	5.95	1.215	0.780
CLA	0.87	0.52	0.43	0.51	0.189	0.456
t-FA/CLA	9.75	14.68	17.85	11.95	3.189	0.415
$C_{18:2}$	10.88	11.46	12.71	16.22	1.046	0.071
$C_{18:3}$	10.49	10.06	10.12	12.08	3.323	0.967
			12 h			
$C_{18:0}$	32.61	33.59	32.65	32.00	1.330	0.863
$C_{18:1}$	19.52	19.01	19.19	17.95	1.109	0.779
t-$C_{18:1}$	12.33	9.84	9.20	7.83	1.968	0.509
CLA	0.82	0.66	0.60	0.54	0.092	0.298
t-FA/CLA	14.94	14.80	15.19	14.54	1.504	0.991
$C_{18:2}$	7.33	9.18	10.98	11.83	1.06	0.128
$C_{18:3}$	8.16	8.76	9.22	12.00	3.224	0.772
			24 h			
$C_{18:0}$	36.64	36.66	36.51	33.85	1.258	0.417
$C_{18:1}$	16.12	16.36	17.74	17.05	0.869	0.597
t-$C_{18:1}$	14.06	12.08	11.56	10.00	1.528	0.416
CLA	0.64	0.67	0.61	0.52	0.072	0.587
t-FA/CLA	21.92	18.06	18.98	19.16	1.286	0.308
$C_{18:2}$	2.80	5.33	7.23	9.13	1.211	0.077
$C_{18:3}$	7.17	6.96	7.41	11.60	0.919	0.469

[1)] Addition levels of concentrate (%, w/v) into 1,200 ml culture solution.
[2)] Means in the same row with different superscripts differ.
[3)] Standard error of the mean.
[4)] Probability levels.
[5)] Total trans-$C_{18:1}$ isomers.
[6)] Conjugated linoleic acid (*cis*-9, *trans*-11 isomer of linoleic acid).
[7)] The ratio of total trans-$C_{18:1}$ isomers to Conjugated linoleic acid.

REFERENCES

Bessa R. J., J. Santos-Silva, J. M. Ribeiro and A. V. Portugal. 2000. Reticulo-rumen biohydrogenation and the enrichment of ruminat edible products with linoleic acid conjugated isomers. Appl. Environ. Microbiol. 63:201-211.

Bryant, M. P. and I. M. Robinson, 1961. An improved nonselective medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446-1454.

Chouinard, P. Y., V. Girard and G. J. Brisson. 1998a. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with varying unsaturation. J. Dairy Sci. 81:471-481.

Chouinard, P. Y., L. Corneau, D. E. Bauman, W. R. Butler, Y. Chilliard and J. K. Drackley. 1998b. Conjugated linoleic acid content of milk from cows fed different sources of dietary fat. J. Anim. Sci. Vol. 76, suppl. 1. J. Dairy Sci. Vol. 81, suppl. 1. (Abstr).

Erasmus, Udo. 1993. Margarines, Shortenings, and *Trans*-fatty acids. Fats that heal, fats that kill. pp. 106-112.

Fawcett, J. K. and J. E. Scott. 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. 13:156-163.

Folch, J., M. Lee and G. H. Sloan-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226:497-509.

Griinari, J. M., D. A. Dwyer, M. A. McGuire and D. E. Bauman. 1996. Partially hydrogenated fatty acids and milk fat depression. J. Dairy Sci. 79(suppl.1):177(Abstr).

Harfoot, C. G. and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. Page 285 in The rumen microbial ecosystem. P.N.Hobson, ed. Elsevier Appl. Sci., London and NY.

Huang, Z. Z., S. H. Choi and M. K. Song. 1999. Metabolic response of ruminal microbes to C_{18}-fatty acids. Korean J. Anim. Nutr. Feed 23:49-58.

Hughes, P. E., W. J. Hunter and S. B. Tove. 1982. Biohydrogenation of unsaturated fatty acids. Purification and properties of cis-9, trans-11-octadecadienoate reductase. J. Biol. Chem. 257:3643-3649.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press. NY.

Jiang J., L. Bjorck and R. Fonden. 1998. Production of conjugated linoleic acid by dairy starter cultures. J. Appl. Microbiol. 85:95-102.

Kelly, M. L. and D. E. Bauman. 1996. Conjugated linoleic acid: A potent anticarcinogen found in milk fat. Proceedings of Cornell Nutr. Conf. Feed Manuf., 58th Cornell Nutrition Conference, Ithaca, NY. pp. 68-74.

Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. V. Amburgh and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881-885.

Kennelly, J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60,137-152.

Kim, Y. J., R. H. Liu, D. R. Bond and J. B. Russell. 2000. Effect of linoleic acid concentration on conjugated linoleic acid production by *Butyrivibrio fibrisolvens* A38. Appl. Environ. Microbiol. 66:5226-5230.

Leat, W. M. F. 1977. Depot fatty acids of Aberdeen Angus and Friesian cattle reared in hay and barley diets. J. Agric. Sci. 89:575-582.

Lepage, G. and C. C. Roy. 1986. Direct transesterification of all classes of lipid in a one-step reaction. J. Lipid Research. 27:114-121.

McDougall, E. I. 1948. Studies on ruminant saliva. 1. The composition and output of sheeps saliva. Biochem. J. 43:99-109.

National Research Council. 1996. Carcinogens and Anticarcinogens in the Human Diet. National Academy of Science, Washington, DC.

Parodi, P. W. 1997. Cows milk fat components as potential anticarcinnogenic agents. J. Nutr. 127:1055-1060.

SAS. 1985. SAS User′s Guide: Statistical Analysis Systems Institute, Inc., Cary, NC.

Scott, H. W. and B. A. Dehority. 1965. Vitamin requirements of several cellulolytic rumen bacteria. J. Bacteriol. 89:1169-1175.

Sehat, N, M. P. Yurawecz, J. A. G. Roach, M. M. Mossoba, J. K. G. Kramer and Y. Ku. 1998. Silver-ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. Lipids 33:217-221.

Solomon, R., L. E. Chase, D. Ben-Ghedalia and D. E. Bauman. 2000. The effect of nonstructural carbohydrate and addition of full fat extruded soybeans on the concentration of conjugated linoleic acid in the milk fat of dairy cows. J. Dairy Sci. 83:1322-1329.

Song, M. K. and S. H. Choi. 1998. Effects of lipid source and addition level on rumen microbial hydrogenation of C_{18}-unsaturated fatty acids and incorporation of oleic acid by rumen bacteria. Korean J. Anim. Sci. 40:31-42.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. Mcgraw Hill Book Co., NY.

Wang, J. H. and M. K. Song. 1999. Effect of dextrose addition levels on hydrogenation and incorporation of oleic acid by ruminal bacteria. Korean J. Anim. Nutr. Feed. 23:335-344.

Wang, J. H. and M. K. Song. 2001. Effect of sources and levels of carbohydrates on fermentation characteristics and hydrogenation of linoleic acid by rumen bacteria *in vitro*. Asian-Aust. J. Anim. Sci. 14:48-53.

Wu, Z., O. A. Ohazuruka and D. L. Palmquist. 1991. Ruminal synthesis, biohydrogenation, and digestibility of fatty acids by dairy cows. J. Dairy Sci. 74:3025-3034.