Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 592–597

Effect of mixed oil on C_{18} - fatty acid and conjugated linoleic acid profiles in rumen fluid and blood plasma of cattle

C.G. Yan[1,3], X.S. Piao[2], Y. Wang[1], H.L. Li[1] and G.J. Xia[1]

[1]Department of Animal Science, Yanbian University
133400 Yanbian, P.R. China
[2]Animal Science and Technology College, China Agricultural University
100094 Beijing, P.R. China

ABSTRACT

The experiment was conducted to determine the effect of mixed oil on C_{18}-fatty acid and CLA profiles in rumen fluid and plasma of Yanbian cattle by adding different levels of the mixed oil to concentrate. The contents of propionate and $C_{18:1}$ were increased in rumen fluid by adding mixed oil. In plasma, the contents of C_{18}-fatty acids in experimental groups were all significantly higher than in the control group; *c9, t11*-CLA increased at 6 h, *t10, c12*-CLA increased at 0, 9, 12 h. Enhancing CLA contents of rumen fluid and plasma through feeding 4% mixed oil in the diet seems to be the optimal level.

KEY WORDS: mixed oil, C_{18}-fatty acid, conjugated linoleic acid, rumen fluid, blood plasma, cattle

INTRODUCTION

Research efforts have been intensified to increase the content of conjugated linoleic acid (CLA) in meat and milk products due to its potential health benefits such as cancer prevention and multiple important physiological effects. It has been reported that an important factor affecting CLA products is the concentration of $C_{18:2}$ in the diet. Supplementation of vegetable oil to the diet has proved to affect the CLA content of ruminant products. We added a mixed oil containing soya bean oil, safflower oil and sunflower oil, all of which are abundant in $C_{18:2}$. This study was conducted to examine the effect of different levels of the mixed oil on C_{18}-fatty acid and CLA profiles in rumen fluid and blood plasma in order to find the optimal supplement level for increasing the contents of CLA in Yanbian cattle.

[3] Corresponding author: e-mail: ycg@ybu.edu.cn

MATERIAL AND METHODS

Three ruminally fistulated Yanbian cattle (body weight, 275±20 kg) were used in an experiment conducted in a 4×3 incomplete Latin square design with 4 periods and 3 dietary treatments in each period. Each period lasted 12 d. The basal diet of each animal included per day, kg: concentrate 4.05, maize silage 6, and rice straw 1. The nutritional composition of the basal diet is presented in Table 1. The diets of experimental groups were supplemented with 4, 5, and 6% (concentrate basis) mixed oil, respectively, added to the concentrate by pouring it on and mixing at feeding to ensure equal distribution in the ration. The control group was not fed the mixed oil. The cattle were fed twice daily (06.30 and 18.30 h). The mixed oil was composed of, %: soya bean oil 55, safflower oil 25 and sunflower oil 20. Proportions (% of total) of palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) as major fatty acids for the mixed oil were 7.65, 3.12, 13.16, 53.27 and 0.31, respectively.

Table 1. The nutritional composition of the basal diet, %, DM basis

Nutrient, %	Concentrate	Rice straw	Maize silage
Crude protein	17. 94	5.19	6.31
Ether extract	5.11	1.17	2.24
NDF	13.57	69.75	46.57
Ash	6.89	6.74	6.47
Ca	1.24	0.62	0.52
P	0.21	0.07	0.10

On day 10, 100 ml rumen fluid was collected from various sites of the rumen at 0, 3, 6, 9 and 12 h post feeding and strained through four layers of gauze. A 0.5 ml aliquot of rumen fluid was mixed with 0.2 ml 25% metaphosphoric acid. The mixture was centrifuged at 10,000 *g* for 10 min at 4°C, and the supernatant was used to determine the composition of VFA by using a Hewlett-Packard HP6890 GC system equipped with a capillary column (HP-INNOWax column, 30m×320 μm×0.50 μm, Hewlett-Packard). The remainder of rumen fluid was stored at -20°C and freeze dried. Fatty acids were analysed according to the method of Raes et al. (2002). The fatty acid methyl esters were analysed by a GC System equipped with a capillary column (DB-23 column, 60 m × 249 μm × 0.25 μm, Hewlett-Packard). The injector and detector temperature was maintained at 250°C, respectively. The initial column temperature was 180°C (held for 10 min), and then increased by 4°C/min to 220°C (held for 15 min). Ultra pure helium was used as the carrier gas. On day 12, 30 ml blood samples were collected from the jugular vein at 0, 3, 6, 9 and 12 h post feeding and then centrifuged immediately at 3,000 *g* for 10 min. The supernatants (plasma) were transferred into 15 ml screw-cap tubes and were kept frozen at -70°C until analysed. Analysis of plasma fatty acids followed the same procedure as that of the rumen fluid analysis.

Data collected from the experiments were subjected to analysis of variance (ANOVA) using the SAS (1996) software. Means were separated by Duncan's multiple range test.

RESULTS

As shown in Table 2, diets supplemented with mixed oil significantly increased the content of propionate (C_3) at each time point ($P \leq 0.0001$), while significantly reducing butyrate (C_4) ($P=0.0002 \sim 0.0010$). However, the total VFA as well as the ratio of acetate to propionate were not affected by dietary supplementation with mixed oil.

Table 2. Concentration of VFA in rumen fluid, mmol/l

VFA	Oil supplement to the diet, %				SEM[1]	P-value[2]
	control	4	5	6		
0 h						
total VFA	77.50	81.21	67.90	72.68	3.192	0.5430
acetate (C_2)	35.50	37.33	30.67	28.50	2.100	0.0001
propionate (C_3)	21.35	25.68	21.89	19.59	1.489	0.0001
butyrate (C_4)	6.70	4.55	3.75	4.77	0.609	0.0002
C_2/ C_3	1.38	1.15	1.12	0.94	0.101	0.5610
3 h						
total VFA	87.42	96.00	82.49	90.97	4.375	0.7910
acetate (C_2)	43.33	48.17	39.33	42.83	2.080	0.0001
propionate (C_3)	28.38	34.86	30.27	32.84	2.258	0.0001
butyrate (C_4)	10.57	8.41	7.05	9.89	1.015	0.0007
C_2/ C_3	1.72	1.38	1.31	1.31	0.100	0.4460
6 h						
total VFA	87.77	89.39	72.07	84.61	4.139	0.4970
acetate (C_2)	45.83	45.50	34.83	40.50	2.527	0.0001
propionate (C_3)	26.89	32.84	27.84	31.76	1.532	< 0.0001
butyrate (C_4)	10.45	6.82	5.00	7.84	1.072	0.0008
C_2/ C_3	1.77	1.39	1.26	1.29	0.114	0.3990
9 h						
total VFA	76.14	81.31	66.30	80.01	3.063	0.3270
acetate (C_2)	40.50	42.17	32.50	39.67	2.025	0.0001
propionate (C_3)	23.24	30.68	25.81	29.59	1.238	0.0001
butyrate (C_4)	8.18	4.43	4.09	6.70	0.819	0.0010
C_2/ C_3	1.78	1.40	1.26	1.35	0.109	0.3850
12 h						
total VFA	60.58	66.09	57.77	58.21	3.478	0.8670
acetate (C_2)	33.00	34.17	28.67	29.83	2.150	0.0001
propionate (C_3)	17.84	23.24	21.89	20.27	1.289	0.0001
butyrate (C_4)	6.25	4.55	3.41	4.66	0.628	0.0004
C_2/ C_3	1.83	1.50	1.33	1.48	0.104	0.4280

[1] standard error of mean; [2] $P<0.05$ differed significantly

The content of $C_{18:1}$ increased by adding the mixed oil (P=0.0001~0.0005) (Table 3). The content of $C_{18:2}$ at 0 h (P=0.0480), 3 h (P=0.0370), 9 h (P=0.0190) and $C_{18:3}$ at 3 h (P=0.0030), 6 h (P=0.0300), and 12 h (P=0.0190) was also enhanced in rumen fluid.

Table 3. Contents of C_{18}-fatty acids in rumen fluid, mg/g of fatty acids

Fatty acid	Oil supplement to the diet, %				SEM[1]	P-value [2]
	control	4	5	6		
0 h						
$C_{18:0}$	7.45	11.53	18.63	24.07	0.344	0.0910
$C_{18:1}$	1.00	1.48	1.38	1.40	0.087	0.0001
$C_{18:2}$	0.63	1.26	0.73	0.79	0.164	0.0480
c9,t11-CLA	0.04	0.12	0.02	0.03	0.026	0.2790
t10,c12-CLA	0.03	0.15	0.09	0.06	0.023	0.3030
$C_{18:3}$	0.11	0.13	0.15	0.23	0.025	0.0600
3 h						
$C_{18:0}$	5.69	7.13	13.47	18.17	0.718	0.1720
$C_{18:1}$	1.21	1.45	1.60	1.63	0.106	0.0002
$C_{18:2}$	0.78	1.49	0.84	1.11	0.170	0.0370
c9,t11-CLA	0.02	0.04	0.02	0.03	0.008	0.1910
t10,c12-CLA	0.01	0.08	0.09	0.15	0.019	0.9340
$C_{18:3}$	0.09	0.12	0.18	0.20	0.015	0.0030
4 h						
$C_{18:0}$	6.95	10.92	13.55	17.88	1.751	0.1160
$C_{18:1}$	1.12	1.69	1.40	1.44	0.109	0.0005
$C_{18:2}$	0.68	1.75	0.75	0.90	0.269	0.1940
c9,t11-CLA	0.03	0.08	0.02	0.02	0.015	0.2680
t10,c12-CLA	0.02	0.08	0.06	0.11	0.019	0.8780
$C_{18:3}$	0.10	0.13	0.18	0.19	0.018	0.0300
9 h						
$C_{18:0}$	6.74	12.09	14.30	21.53	2.197	0.3560
$C_{18:1}$	1.04	1.27	1.24	1.44	0.064	0.0001
$C_{18:2}$	0.64	0.93	0.59	0.72	0.102	0.0190
c9,t11-CLA	0.03	0.07	0.02	0.02	0.013	0.2520
t10,c12-CLA	0.05	0.09	0.03	0.10	0.128	0.0570
$C_{18:3}$	0.10	0.13	0.18	0.22	0.021	0.1020
12 h						
$C_{18:0}$	7.45	11.86	15.74	23.94	2.353	0.1400
$C_{18:1}$	1.07	1.58	1.33	1.40	0.111	0.0004
$C_{18:2}$	0.71	1.41	0.61	0.69	0.223	0.0940
c9,t11-CLA	0.04	0.13	0.02	0.02	0.028	0.3270
t10,c12-CLA	0.03	0.16	0.05	0.07	0.028	0.3980
$C_{18:3}$	0.11	0.12	0.19	0.23	0.020	0.0190

[1] standard error of mean; [2] $P<0.05$ differed significantly

In blood plasma (Table 4), the contents of $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ in experimental groups were all significantly higher than in the control group (P=0.0080~0.0220; P=0.0050~0.0420; P=0.0020~0.0070; P=0.0001~0.0007). The contents of *c9, t11*-CLA increased in experimental groups at 6 h (P=0.0320), and *t10, c12*-CLA increased at 0, 9 and 12 h (P=0.0030~0.0110).

Table 4. Contents of C_{18}-fatty acids in blood plasma, mg/g fatty acids

Fatty acid	Oil supplement to the diet, %				SEM[1]	P-value[2]
	control	4	5	6		
0 h						
$C_{18:0}$	1.59	2.60	2.51	2.87	0.286	0.0090
$C_{18:1}$	0.93	1.31	1.40	1.28	0.142	0.0050
$C_{18:2}$	3.47	6.54	4.96	5.91	0.546	0.0030
c9, t11-CLA[3]	46.53	37.55	28.34	21.88	0.007	0.0040
t10, c12-CLA[3]	4.99	8.67	6.02	7.87	0.009	0.0090
$C_{18:3}$[3]	14.23	20.58	19.96	18.62	0.002	0.0007
3 h						
$C_{18:0}$	1.35	2.58	2.29	2.75	0.277	0.0220
$C_{18:1}$	0.84	1.33	1.34	1.29	0.136	0.0090
$C_{18:2}$	3.42	6.40	4.71	5.99	0.558	0.0070
c9, t11-CLA[3]	15.85	28.42	26.43	63.03	0.011	0.6770
t10, c12-CLA[3]	5.43	9.25	7.17	11.69	0.002	0.1210
$C_{18:3}$[3]	13.56	20.15	18.60	18.06	0.001	0.0001
6 h						
$C_{18:0}$	1.42	1.91	2.22	2.70	0.215	0.0090
$C_{18:1}$	0.90	0.81	1.33	1.27	0.157	0.0420
$C_{18:2}$	3.27	4.40	4.72	6.04	0.425	0.0030
c9, t11-CLA[3]	15.99	24.60	25.98	25.09	0.003	0.0320
t10, c12-CLA[3]	4.46	12.75	6.99	6.34	0.001	0.0770
$C_{18:3}$[3]	14.82	16.32	18.10	17.94	0.001	0.0001
9 h						
$C_{18:0}$	1.57	1.95	2.27	2.55	0.203	0.0080
$C_{18:1}$	0.90	1.13	0.39	1.16	0.137	0.0220
$C_{18:2}$	3.29	4.99	4.69	5.80	0.390	0.0020
c9, t11-CLA[3]	13.84	25.03	19.01	18.94	0.003	0.0940
t10, c12-CLA[3]	5.69	7.75	7.50	6.67	0.001	0.0030
$C_{18:3}$[3]	18.14	17.70	20.46	16.79	0.001	0.0001
12 h						
$C_{18:0}$	1.75	2.14	2.39	2.85	0.238	0.0110
$C_{18:1}$	0.93	1.08	0.70	0.96	0.154	0.0280
$C_{18:2}$	3.64	4.82	4.64	6.11	0.458	0.0060
c9, t11-CLA[3]	9.26	31.52	22.65	20.53	0.004	0.1790
t10, c12-CLA[3]	5.40	7.93	5.84	8.18	0.001	0.0110
$C_{18:3}$[3]	18.16	20.37	19.58	18.83	0.001	0.0001

[1] standard error of mean; [2] P<0.05 differed significantly; [3] ($\times 10^{-3}$) mg/g

DISCUSSION

Jenkins and Jenny (1989) indicated that supplementation of lipid to the diet decreased the C_2 while increased the C_3 percentage due to reduced degradation of fibre in the rumen. In this study, we found that the contents of C_3 were all increased at different collection times because of the supplemented mixed oil. Kim et al. (2000) indicated that rumen bacteria produced greater amounts of CLA when the concentration of $C_{18:2}$ in the diet was high enough to reduce bacterial growth. An important factor affecting CLA production is the concentration of dietary $C_{18:2}$ (Chouinard et al., 1998; Kelly et al., 1998). Our results showed that supplementing mixed oil reduced the hydrogenation rate of C_{18}-unsaturated fatty acids by rumen bacteria. The content of $C_{18:1}$ was significantly increased both in rumen fluid and plasma of the experimental groups, therefore, it could provide abundant raw materials for synthesizing CLA. The contents of $C_{18:2}$ and $C_{18:3}$ both in the rumen fluid and plasma of each experimental group tended to be higher than in the control group, similarly as the contents of *c9,t11*-CLA and *t10,c12*-CLA. But the contents of C_{18}-unsaturated fatty acids and CLA in rumen fluid and blood plasma did not increase with the rising levels of the mixed oil. As shown in the tables, the contents of propionate, C_{18}-unsaturated fatty acids and CLA both in rumen fluid and blood plasma of group 4% were highest among all groups. This might be related to the weight of the cattle.

CONCLUSIONS

Based on our results, we conclude that a supplement of 4% mixed oil was the optimal dietary level for Yanbian cattle weighing 275 kg because it increased the contents of C_{18}-unsaturated fatty acids and CLA isomers both in rumen fluid and blood plasma.

REFERENCES

Chouinard P.Y., Corneau L., Bauman D.E., Butler W.R., Chilliard Y., Drackley J.K., 1998. Conjugated linoleic acid content of milk from cows fed different sources of dietary fat. J. Anim. Sci. 76, Suppl. 1, 233 (Abstr. 906)

Jenkins T.C., Jenny B.F., 1989. Effect of hydrogenated of fat on feed intake, nutrient digestion and lactation performance of dairy cows. J. Dairy Sci. 72, 2316-2324

Kelly M.L., Kolver E.S., Bauman D.E., Van Amburgh M.E., Muller L.D., 1998. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk form lactating cows. J. Dairy Sci. 81, 1630-1636

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

Raes K., Huyghebaert G., De Smet S., Nollet L., Arnouts S., Demeyer D., 2002. The deposition of conjugated linoleic acid in eggs of laying hens fed diets varying in fat level and fatty acid profile. J. Nutr. 132, 182-189

SAS, 1996. SAS User's Guide: Statistics. Version 7.0. SAS Institute Inc. Cary, NC