PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 12 (6): 516-520, 2013
ISSN 1680-5194

Effect of Dietary Vegetable Oil Supplementation on C_{18} Fatty Acids and Conjugated Linoleic Acid Production; An *In vitro* Fermentation Study

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Abstract: An *in vitro* rumen fermentation trial was conducted to investigate effect of dietary vegetable oil supplement on fatty acids metabolism in cattle. The incubation was carried out using rumen fluid obtained from Brahman x Native crossbred beef cattle. The diets were formulated by using basal diet with no vegetable oil (CON), basal diet with 4% of palm oil (PO), 4% of canola oil (CA), 4% of coconut oil (CO) and 4% of sunflower oil (SF). Fatty acid profiles were determined at 12, 24 and 48 h after incubation time. The results found that linoleic acid (LA) was decreased with diet CON, diet PO, diet CA and diet SF ($p<0.05$). Stearic acid (SA) resulted increased with diet CON, diet CA, diet CO and diet SF at 24 and 48 h. The SF diet contain high level of LA showed the highest concentration of cis9 trans11 conjugated linoleic acid (CLA) when compared with other diets. Vaccenic acid (VA) was increased with all the five diet, especially with diet SF and diet CA after 24 h. Base on this study, it suggests that supplementation of SF could improve the concentration of VA and cis9 trans11 CLA in the rumen.

Key words: Vegetable oil, biohydrogenation, fatty acids, rumen fermentation

INTRODUCTION

Vegetable oil is typically added in animal feed to increase an energy source of the ration, increase absorption of fat-soluble nutrients and reduced dustiness of the diet (NRC, 2001). Moreover, vegetable oil are usually used as sources of unsaturated fatty acids, mostly linoleic acid to increase the concentration of biohydrogenation intermediates especially CLA isomer and VA (Khanal and Dhiman, 2004). These fatty acids will be transferred to mammary gland and to other tissues (Jenkins, 1993). Previous researches reported that the influence of the diet on concentration of CLA in meat and milk as the consequence of different profile of VA and CLA isomer during rumen fermentation (Griinari and Bauman, 1999; Kramer *et al*., 1999). The studies of rumen biohydrogenation processes with different type of vegetable oils in the diet should be done to determine the accumulation of CLA and VA fatty acids composition in ruminal fluid to increase the level of both fatty acids in animal products.

Types of vegetable oil supplements could affect the processes of biohydrogenation and consequently, the final products. Several researches showed that LA, Linolenic Acid (LNA) lead to an accumulation of CLA and VA in the rumen. However, recently data showed that oleic acid has been proposed as a rumen precursor of VA. It also could be attributed to increase the level of CLA in the rumen and other tissues (Selner and Schultz, 1980; Mosley *et al*., 2002). Panyakaew *et al*. (2013) reported the positive effect of coconut oil on the biohydrogenation processes by inhibit the last hydrogenation step converting trans $C_{18:1}$-$C_{18:0}$ when compared with no supplemental coconut oil.

Therefore, the aim of this study was to evaluate effect of diet with different vegetable oil on the concentration of C18 fatty acids and CLA isomers during *in vitro* rumen fermentation.

MATERIALS AND METHODS

All experimental cattle and procedures were managed according to the guidelines approved by the Animal Ethic Committee of Khon Kaen University (No. AEKKU 05/2555). Prior to experiments, all cattle were examined remained healthy throughout study.

Experimental design and fermentation technique: The experiment was conducted using an *in vitro* rumen fermentation technique at various incubation time intervals with three replications per treatment. The vegetable oil (Palm Oil (PO), canola oil (CA), Coconut Oil (CO) and sunflower oil (SF)) was supplemented in the diets and the diet with no supplemental vegetable oil was used as a control treatment. The diets used for these substrates were 70% concentrate and 30% rice straw as a roughage source. Feed ingredients and nutritive value is shown in Table 1. All substrates were

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Table 1: Ingredients and chemical composition of basal diet

Item	Basal diet
Ingredient (%)	
Rice straw	30.0
Cassava chip	22.0
Ground corn	12.0
Palm kernel	5.0
Soybean meal	17.0
Urea	1.0
Molasses	1.0
Mineral premix	3.0
Salt	1.0
Total	100.0
Chemical composition	
DM (%)	91.25
	--% DM --
OM (%)	93.32
CP (%)	12.48
Ether extract (%)	1.77
Gross Energy (Mcal/kg)	3.46

Table 2: Fatty acid composition (%FAME) of diets with different vegetable oil

	Diet				
	CON	PO	CA	CO	SF
C8:0 Caprylic	0.12	0.00	0.00	2.14	0.00
C10:0 Capric	0.04	0.00	0.00	4.26	0.00
C12:0 Lauric	1.55	0.02	0.13	48.52	0.17
C14:0 Myristic	0.32	1.32	0.00	19.63	0.06
C16:0 Palmitic	12.65	38.92	4.12	9.02	5.64
C18:0 Stearic	2.82	4.83	1.98	4.39	5.52
C18:1n-9 oleic	16.82	47.12	64.38	8.57	38.78
C18:2n-6 linoleic	28.12	11.65	24.12	2.06	54.36
C18:3n-3 α-linolenic	2.65	0.06	13.54	0.08	1.08

Note: PO mean palm oil, SF mean sunflower oil, CA mean canola oil, CO mean coconut oil and CON mean control diet

ground pass through 1-mm screen. Substrates (500 mg of DM) was added into 120 ml bottles and supplemented with difference vegetable oils. Diet samples were incubated for various incubation times. Feed samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and chemically analyzed using the standard methods of AOAC (1995) for dry matter (DM, ID 967.03), ash (ID 942.05) and acid-detergent fiber (ADF, ID 973.18). Neutral-detergent Fiber (NDF) in samples was determined according to Van Soest *et al.* (1991). Total nitrogen in samples of feeds was determined according AOAC (1991) (ID 984.13).

Two, rumen fistulated (Brahman x Native crossbred cattle with an initial BW of 448±13 kg) were used as rumen fluid donor. The rumen fluid (1,000 ml) was collected from cattle fed with concentrate and roughage at the ratio of 70:30 (14.0% CP and 2.4 Mcal/kg, dry matter basis) before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks add with artificial saliva which prepare according to Menke and Steingass (1988). Fluid was made by the ratio of artificial saliva: rumen fluid at 2:1 (v/v). The bottles with the mixture of substrate treatments were pre-warmed in a hot air oven at 39°C for 24 h. before filling with 80 ml of rumen inoculum's mixture. The inoculums were fermented for 12, 24 and 48 h according to Beam *et al.* (2000), Mosley *et al.* (2002) and Baccioni *et al.* (2006) and pH was monitored throughout the fermentation period (12, 24 and 48 h).

Extraction of fatty acids: The rumen fluid sample was freeze-dried (Heto PowerDry LL3000 Freeze Dryer; Thermo Fisher Scientific, Tehovec-Mukarov, Czech Republic) and 1 g of freeze-dried material was extracted with 10 ml of a mixture of chloroform and methanol (2:1) according to the modificated method of Folch *et al.* (1957), The mixture was stirred for 30 min at laboratory temperature. This mixture was transferred to separation funnel and kept the lower layer (i.e., chloroform layer with lipids). The upper layer was extracted again with 10 ml of a mixture of chloroform and methanol (2:1). The lower layer was kept and added to the first filtered. To the filtered mixture, 1.5 ml of 6N HCL was added and vortex for 5 min. The mixture was centrifuged at 200 x g for 5 min, at laboratory temperature, the upper layer was removed and the bottom phase was dried in water bath at 50°C under N_2 for 15 min.

Fatty acid analysis: Extracted lipids were saponified and methylated according the procedure of Metcalfe *et al.* (1966) with Nonadecanoic acid (Fluka Chemie GmbH, Buchs, Switzerland) as the internal standard. Fatty acid methyl esters were determined using gas chromatography. Samples were injected by split less injector in to Perkin-Elmer Clarus 500 gas chromatograph (Perkin-Elmer, Inc., Sherlton, CN, USA) equipped with a CP-Sil88 Column for fatty acid methyl esters (FAME; 100m x 250 µm x 0.2 µm, Chrompack, Middelburg, The Netherlands). Fatty acid peaks were identified base on their retention time and compared with the commercial standard mixture and published isomeric profile (Wolff and Bayard, 1995). All fatty acids composition results are expressed as % of lipid extract.

Statistical analysis: Data were analyzed by using the General Linear Models (GLM) procedures (SAS Inst. Inc., Cary, NC) with two factors with interaction: diet and fermentation time. Data analyzed using the model:

$$y_{ij} = \mu + D_i + T_j + D_iT_j + e_{ij}$$

where, y_{ij} is the observation; μ the overall mean; D_i the diet ($_i$ = 1, 2, 3, 4); T_j the fermentation time ($_j$ = 1 - 3); D_iT_j the interaction between diet and fermentation time and e_{ij} the residual error. Means of all comparisons means

were compared according to Duncan's New Multiple Range Test, DMRT (Steel *et al*., 1997).

RESULTS

C_{18} isomer profile: The effect of vegetable oil supplement on the concentration of C_{18} isomer fatty acid profile (% lipid extract) is shown in Table 3. The concentration of SA was increased with diet CON, diet CA, diet CO and diet SF at 24 h and 48 h after incubation but, the concentration of SA were not significantly different ($p>0.05$) with diet PO at 24 h and 48 h after incubation. The concentration of VA was increased with all the five diet, especially with diet SF and diet CA after 24 h. Finally, the linoleic acid (C18:2) were rapidly decrease ($p<0.05$) at all incubation time in diet SF, diet CA and diet PO, excepted in diet CO. Linolenic acid ($C_{18:3}$) concentration were slightly decrease following the fermentation time in all diets excepted in diet CO.

Table 3: Effect of vegetable oils supplement on C_{18} fatty acids at different fermentation time (% of lipid extract) (means of three replication)

	Fermentation times (h)			
Item	12	24	48	SEM
$C_{18:0}$				
Diet CON	5.33^{a}	6.03^{Ab}	10.25^{Ac}	0.78
Diet PO	4.26^{a}	6.48^{Ab}	7.59^{Bb}	
Diet CA	5.87^{a}	7.46^{Bb}	12.75^{Cc}	
Diet CO	4.98	5.26^{A}	6.23^{D}	
Diet SF	5.83^{a}	5.94^{Aa}	9.83^{Ab}	
C18:1n9c				
Diet CON	6.59^{Aa}	5.63^{Aa}	3.56^{Ab}	1.16
Diet PO	9.23^{Ba}	7.78^{Aa}	5.23^{Ab}	
Diet CA	11.46^{Ca}	12.03^{Baa}	8.21^{Bb}	
Diet CO	2.23^{D}	2.02^{C}	1.56^{C}	
Diet SF	16.12^{Ea}	7.89^{Ab}	11.06^{Dc}	
C18:1n9t				
Diet CON	-	-	0.02	0.002
Diet PO	-	-	0.02	
Diet CA	-	-	0.01	
Diet CO	-	-	-	
Diet SF	-	-	0.03	
C18:1n11t				
Diet CON	1.32^{Aa}	4.97^{Ab}	6.83^{Ab}	1.08
Diet PO	0.56^{ABa}	6.37^{Ab}	8.66^{Ac}	
Diet CA	2.22^{ACa}	9.42^{Bb}	11.77^{Bb}	
Diet CO	0.31^{Aa}	2.28^{Ca}	3.87^{Cc}	
Diet SF	2.56^{Ca}	10.45^{Db}	14.23^{Dc}	
C18:2 c9c12				
Diet CON	12.94^{Aa}	8.12^{Ab}	3.62^{Ac}	0.78
Diet PO	18.15^{Ba}	11.54^{Bb}	8.89^{Bc}	
Diet CA	24.44^{Ca}	14.87^{Cb}	11.84^{Cc}	
Diet CO	1.36^{D}	0.78^{D}	0.89^{D}	
Diet SF	36.72^{Ea}	15.68^{Eb}	13.82^{Ec}	
C18:3 c9c12c15				
Diet CON	2.74^{Aa}	0.62^{Ab}	0.45^{Ab}	0.65
Diet PO	1.23^{A}	0.98^{A}	1.12^{AC}	
Diet CA	6.53^{Bs}	2.16^{Bb}	1.87^{BCb}	
Diet CO	-	0.12^{A}	0.14^{A}	
Diet SF	1.46^{A}	0.85^{A}	0.47^{A}	

Note: Means with different capital letters (A, B, C, D) within the same column are significantly different ($p<0.05$); Means different with small letters (a, b, c) within the same row are significantly different ($p<0.05$)

Conjugated $C_{18:2}$ isomer profile: The effect of vegetable oil supplementation on the concentration of conjugated $C_{18:2}$ isomer fatty acid profile (% lipid extract) is shown in Table 4. All of the conjugated $C_{18:2}$ isomer at 12 h cannot detected for diet PO and diet CO and slightly detected in diet CON, diet CA and diet SF. The concentration of cis9 trans11 CLA and total CLA in SF diet showed the greater amount at 24 and 48 h ($p<0.05$) more than other diets.

DISCUSSION

In this studied was conducted to determine the four types of vegetable oil were supplemented in the diet on the fatty acid composition of an *in vitro* rumen fermentation. The fatty acids profiles of the diets were shown in Table 2 with aim on the fatty acid composition: palmitic acid were higher in diet PO; linoleic acid were higher in diet SF; oleic acid were higher in diet CA and lauric acid were higher in diet CO.

Table 4: Effect of vegetable oils supplement on conjugated $C_{18:2}$ fatty acids at different fermentation time (% of lipid extract) (mean of three replicates)

	Fermentation times (h)			
Item	12	24	48	SEM
Cis 9 Tran 11 $C_{18:2}$				
Diet CON	0.18	0.26^{A}	0.17^{A}	0.12
Diet PO	-	0.28^{A}	0.14^{A}	
Diet CA	0.08^{a}	0.64^{Bb}	0.48^{Ab}	
Diet CO	-	0.04^{c}	0.09^{A}	
Diet SF	0.22^{a}	1.21^{Db}	1.42^{Bb}	
Trans 10 Cis 12 $C_{18:2}$				
Diet CON	0.03	0.12^{A}	0.05^{A}	0.09
Diet PO	-	0.08^{A}	0.07^{A}	
Diet CA	0.02^{a}	0.09^{Ab}	0.13^{Ab}	
Diet CO	-	-	0.02^{A}	
Diet SF	0.01^{a}	0.19^{Bb}	0.36^{Bc}	
Trans 9 Trans 11 $C_{18:2}$				
Diet CON	-	0.11^{Aa}	0.07^{b}	0.02
Diet PO	-	0.04^{Ba}	0.08^{b}	
Diet CA	-	0.18^{Aa}	0.07^{b}	
Diet CO	-	0.02^{B}	-	
Diet SF	-	0.27^{Ca}	0.12^{b}	
Other CLA				
Diet CON	0.001^{Aa}	0.021^{Ab}	0.003^{Ac}	0.004
Diet PO	-	0.021^{Aa}	0.016^{Bb}	
Diet CA	0.004^{Aa}	0.014^{Ab}	0.017^{Bb}	
Diet CO	-	0.00^{dB}	0.003^{A}	
Diet SF	0.002^{Ba}	0.023^{Ab}	0.017^{Bb}	
Total CLA				
Diet CON	0.23^{Aa}	0.51^{Ab}	0.29^{Aa}	0.11
Diet PO	-	0.42^{A}	0.31^{A}	
Diet CA	0.12^{Ba}	0.92^{Bb}	1.19^{Bb}	
Diet CO	-	0.06^{c}	0.11^{c}	
Diet SF	0.23^{Aa}	1.67^{Db}	1.82^{Db}	

Note: Means with different capital letters (A, B, C, D) within the same column are significantly different ($p<0.05$); Means different with small letters (a, b, c) within the same row are significantly different ($p<0.05$)

The most intensive changes in fatty acid concentrations, including formation of CLA isomer, occur in the first 24 h of incubation. One of the most important factors for the biohydrogenation process in the rumen is the ruminal pH that depends on the substrate type and concentration (Szumacher-Strabel *et al*., 2009). The incubation pH in this studied was monitored and the value were stable about 6.8 for all incubation. These data are supported by previous studies which suggested that the pH is the most important factor for the biohydrogenation process in the rumen. Ruminal pH at 6.0 or above has a positive effect on VA and CLA isomer (Martin and Jenkins, 2002; Troegeler-Meynadir *et al*., 2003).

Fat in ruminant diets can change biohydrogenation of unsaturated fatty acids in the rumen. The main end product of biohydrogenation are SA. The result of this study showed the lower concentration of SA was due to an incomplete biohydrogenation process (Jalc *et al*., 2005) and thus increase levels of intermediates, such as VA and CLA isomer, were observed. Baccioni *et al*. (2006) reported that the first stage of rumen biohydrogenation was the isomerization from LA to cis9 trans11 CLA which actually was significantly increased to a maximum around 36 h whereas VA increase all the time. The second state is the biohydrogenation down to VA, the trans 11 isomer of $C_{18:1}$. The cis9, trans11 CLA reached its maximum around 36 h and then declined. The final stage lead to SA (Jenkins, 1993; Bauman *et al*., 2003; AbuGhazaleh *et al*., 2003). In our experiment, SF diet showed the higher concentration of cis9, trans11 CLA when compared with the other diets. These data were in agreement with Matsushita *et al*. (2007), who compared the supplementation of sunflower oil, soybean oil and canola oil in goats diet and found cis9 trans11 CLA concentrations in milk were increased. The effect of fat on rumen fermentation depends on the type of fat, mostly on fatty acid saturation. The occurrence of $C_{18:1}$ and $C_{18:2}$ isomers depends on the dietary fatty acid profile (Loor *et al*., 2002).

Conclusions: It can be concluded that, a supplementation of sunflower oil in the diet can increase VA and cis9 trans11 CLA concentration more than other treatment at 24 and 48 h after incubation.

ACKNOWLEDGEMENTS

The authors would like to express their most sincere thanks to the Office of the Higher Education Commission, Thailand, for supporting by grant fund under the program Strategic Scholarships for Frontier Research Network for the Joint Ph.D. program Thai Doctoral degree, and also thank to the supporting from The Increased Production Efficiency and Meat Quality of Native Beef and Buffalo Research Group, Khon Kaen University for this research.

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