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Effect of vegetable oil source supplementation on feed intake, nutrients digestibility and rumen biohydrogenation bacterial population in Thai Friesian dairy cows

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ABSTRACT

The investigation aimed to study the effect of vegetable oil sources on feed intake, nutrient digestibility and biohydrogenation bacterial population in Thai Friesian dairy cows. Three fistula Thai Friesian cows with mean body weight (BW) of 600±100 kg were assigned to receive three vegetable oil sources i.e. palm oil (PO), soybean oil (SBO) and sunflower oil (SFO) included at 4 %DM in commercial concentrate. All cows were fed on different diets that contained concentrate of 1 %BW and rice straw of 1 %BW according to a 3×3 latin square design (LSD). The results revealed that dry matter digestibility (DMD) and organic matter digestibility (OMD) tended to be higher in cows fed on SBO and SFO (P=0.06). Moreover, the DNA copy numbers (copies/ml) of biohydrogenation bacteria (*Ruminococcus albus*) and cellulolytic bacteria *(Ruminococcus flavefaciens* and *Prevotella ruminicola*) were higher in cows fed on SBO (P<0.05). In summary, supplementation of SBO in diet resulted in a higher nutrient digestibility and rumen biohydrogenation bacteria population.

Key words: Biohydrogenation bacteria, Cow, Digestibility, Vegetable oil.

INTRODUCTION

Ruminant animals are capable of digesting structural carbohydrate (SC) presented in plants through fermentation by rumen microorganisms (Krause *et al*., 2003). High levels of lipids in the diet could adversely affect on rumen microbial population (Chilliard *et al*., 2000). Van Soest (1994) described that when lipids containing higher proportions of polyunsaturated fatty acid (PUFA) were provided as an energy source and they affected the permeability of the microbial membrane. In particular, PUFA can manipulate rumen fermentation by inhibiting activity and growth of ruminal bacteria. More recent studies had identified *Anaerovibrio lipolytica*, *Butyrivibrio fibrisolvens*, *B. proteoclasticus*, *Ruminococcus albus* and *Megasphaera elsdenii* as the principal rumen bacteria in the biohydrogenation of C:18 unsaturated fatty acids (Harfoot and Hazlewood, 1997; Kim *et al*., 2002; Wallace *et al*., 2006; Paillard *et al*., 2007).

Recent studies suggested that supplementation of palm oil (PO) at 8 % dry matter (DM) in the diet decreased the abundance of *R. albus* in Nellore steers (Carvalho *et al.*, 2017). Furthermore, supplementation of soybean oil (SBO) at 6 %DM in the diet decreased the population of *R. flavefaciens*, *R. albus* and *Fibrobacter succinogenes* in Nellore steers (Granja-Salcedo *et al*., 2017). Moreover, supplementation of sunflower oil (SFO) at 6 %DM in the diet had no effect on dry matter digestibility (DMD), neutral detergent fiber digestibility (NDFD) and crude protein digestibility (CPD) in Thai native cattle (Polviset and Prakobsaeng, 2016). On the other hand, Zhao *et al*. (2016) suggested that supplementation of SFO (40 g/kgDM) in the diet decreased *B. proteoclasticus* in lambs.

Hence, the effect of lipids on rumen fermentation depends on the composition of lipids chemicals and the level of inclusion of lipids in the diet (Jenkins *et al*., 2008). Therefore, the purpose of this experiment was to investigate the effect of supplementation PO, SBO and SFO at 4%DM in the concentrate of fistula Thai Friesian cows (crossbred Holstein Friesian) fed on rice straw as a basal roughage source on feed intake, nutrient digestibility, rumen fermentation and rumen biohydrogenation bacteria population.

MATERIALS AND METHODS

Animal design and treatments: Three fistula Thai Friesian cows (600±100 kg BW) were used in 3x3 latin square design (LSD) and randomly allocated to receive three sources of vegetable oil (PO, SBO and SFO) added at 4 %DM in concentrate (12 %CP). Fistula cows were received rice straw as a roughage source and fed on their respective treatment diets at 2% of their body weight for maintenance (1 %BW of commercial concentrate and 1% BW of rice straw). Freshwater and mineral block were offered as free choices.

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Sample collecting: Feed offered and feed refused were weighed daily for measuring feed intake. Feed and fecal samples were randomly collected for analysis of DM, organic matter (OM), ether extract (EE), crude protein (CP), Ash (AOAC, 1990), Neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest *et al*., 1991). Samples were analyzed apparent digestibility according to Van Keulen and Young (1977). The rumen fluid sample was collected at 0, 2 and 4 h-post feeding using rumen stomach tube and vacuum pump. The rumen fluid sample was divided into two portions, the first portion was filtered through four layers of cheesecloth and added 10 ml of 1M H_2SO_4 solution for every100 ml of rumen fluid. The supernatant was separated after centrifuging at 16,000 x *g* for 15 minutes and stored at -20°C prior to NH_3-N measurement (Bremner and Keeney, 1965) and VFA analysis (Mathew *et al*., 1997). The second portion of rumen fluid sample was collected 2 h-post feeding and stored at -20°C until bacterial analysis (Gudla *et al*., 2012). The frozen rumen fluid samples were thawed at room temperature and the community DNA was extracted from 500 µl of rumen fluid content by the RBB+C method (Yu and Morrison, 2004). The concentration of the genomic DNA was determined with a Nanodrop Spectrophotometer. The targeted rumen bacteria included total bacteria, *B. fibrisolvens, B. proteoclasticus, A. lipolytica, M. elsdenii, R. Albus, R. Flavefaciens, F. succinogenes* and *Prevotella ruminicola*. The Species-specificity of 16s DNA gene primers used in this experiment were specified by Wongnen (2016) (Table 1). The primers were confirmed by using in-silico techniques with the BLAST program in the Gene-Bank Database of National Center for Biotechnology Information (NCBI). The ruminal bacteria were determined by real-time PCR analysis and used fluorescence detection of SYBR green mix described by Potu *et al*. (2011). At the same time of rumen fluid sampling, blood samples were drawn from the jugular vein. Serum was separated by centrifugation at 500 ×*g* for 10 minutes and then stored at –20°C until blood urea nitrogen (BUN) analysis according to Crocker (1967).

Statistical analysis: The data were analyzed according to a 3x3 latin square design by Analysis of Variance (ANOVA) using the General Linear Model Procedure (GLM) of the SAS 1996. Treatment means were compared by using Duncan's New Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The chemical composition of the diets: Diets contained low CP, high NDF and ADF (Table 2). The appropriate level of NDF in dairy cattle diet should be 35 % (Mertens, 1997), while the NRC (2001) recommends that dairy feeds should have at least 25-35 %NDF and not less than 19-21 %ADF. Moreover, the amount of fat in the total diet was 3.48 %, which was under the appropriate level of 2-5 % (Church, 1979).

Feed intake and nutrient digestibility: The feed intakeand nutrient intake were not different among treatments (P>0.05). However, in cows given SFO and SBO the DMD and OMD tended to be higher than that in cows given PO (P≤0.06) (Table 3). Likewise, the estimated energy intake (MEI) in cows that received SFO and SBO tended to be higher than PO (P≤0.08). Shingfield *et al*. (2011) reported that adding

Table: 1 Species-specific rumen bacteria primers used in this experiment (Wongnen, 2016).

Target bacterial	Primer sequences (5'–3')	Annealing Temp. (°C)	Product size (bp)	r^2
Total Bacteria	[1]F-CGGTGAATACGTTCYCGG	60	124	0.990
	[2]R-GYYACCTTGTTACGACTT			
Bio-hydrogenation bacteria				
Butyrivibrio fibrisolvens	F- AAAGCTCTATCAGCAGGGAA	55.2	126	1.000
	R- GTAAATCCGGATAACACTTG			
Butyrivibrio proteoclasticus	F- GGGATATTGCACAATGGAGG	56	106	0.992
	R- TCTCTTGCGAGCCTTTCTTC			
Anaerovibrio lipolytica	F- CTAGTGGCAAACGGGTGAGT			
	R- GCCTTTGGTACATCGGTCAT	52	120	0.998
Megasphaera elsdenii	F- AGGACAAGAAAACAGGTGG			
	R- CGCTGGTAACAGAAGATAGG	50.8	103	0.998
Ruminococcus albus	F- GCTTACTGGGCTTTAACTGA			
	R- CCCACACCTAGTAATCATCG	55	103	0.999
Cellulolytic bacteria				
Ruminococcus flavefaciens	F- GTAGCCGGACTGAGAGGTTG			
	R- ATCGCTGCATCAGGGTTTC	56.9	113	0.999
Fibrobacter succinogenes	F- CAACCCACGTTTCCAGTT			
	R- TGTGTAGCCCAGGATGTAA	55	119	0.994
Prevotella ruminicola	F- GGAAGTCTGAACCAGCCAAG			
	R- TACCTACAAACGGGGACACG	53.7	103	0.999

[1]Forward Primer from Suzuki *et al*. (2000), [2]Reverse Primer from Nicol *et al*. (2008).
Temp. = Temperature, F = forward, R = reverse, r^2 = R-Square and bp = base pair.

oil in animal feeds below 4 %DM had no effect on feed intake and nutrient digestibility. Similarly, Polviset *et al.* (2014) reported that the different fat sources at 6 % DM in TMR diet did not affect feed intake. In this trial, feed intake was below the level of feeding at 2 % BW. This may be due to the reason that the experimental diet contained high NDF; the amount of feed intake had been limited by NDF content in the feed (Krause *et al.*, 2003). Furthermore, high-level supplementation of lipids in experiment concentrate had a negative effect on decreased rumen degradation and cellulolytic bacteria population in the rumen (Wanapat, 1990).

Rumen fermentation patterns and blood metabolites: The rumen pH, NH_3-N, BUN and VFAs were not significantly different among treatments (P>0.05) (Table 4). The concentration of ruminal NH_3-N was lower (9.45-10.80 mg %), direct variation with a decrease in the lower feeding, and its availability could have been used in microbial protein synthesis. The appropriate level of rumen NH_3-N for growth and microbial activity was in the range 5-25 mg% (Preston and Leng, 1987). The concentration of BUN was lower (5.54-6.89 mg%), the concentration of BUN correlated with the level of NH_3-N in the rumen fluid (Paengkoum, 1998). The concentration of volatile fatty acid (VFA) was not different among treatments (P<0.05). The proportion of VFA depended on the type of feed and ratio of roughage to concentrate (Wachirapakorn, 1998).

Rumen biohydrogenation bacterial population: DNA copy numbers (copies/ml) of biohydrogenation bacteria (*R. albus*) and cellulolytic bacteria (*R. flavefaciens* and *P. ruminicola*) were higher in cows fed on SBO than that in other treatments (P<0.05) (Table 5). DNA copy number of

Table 2: Chemical composition (% DMB) of concentrate and roughage fed to experimental animals.

		Concentrate			
Items	**Concentrate**	**Rice Straw**	**Palm**	**Soybean**	**Sunflower**
Dry matter (DM)	96.00	94.50	95.50	95.50	95.50
Chemical composition (% DM basis)					
OM	91.75	88.75	92.25	92.75	92.25
CP	11.94	2.25	11.47	11.47	11.53
EE	2.34	0.72	6.09	6.87	6.07
NDF	21.67	75.00	23.33	24.16	22.50
ADF	10.00	49.17	10.83	11.67	11.67
ADL	5.00	5.00	4.17	5.00	3.33
Ash	11.32	11.25	7.75	7.25	7.75

OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber and ADL = acid detergent lignin.

Table 3: Effect of vegetable oil on voluntary feed intake and nutrient digestibility coefficient.

Items	**Palm**	**Soybean**	**Sunflower**	**SEM**	**P-value**
Body Weight (kg)	624.58	617.82	623.17		
DM intake (kg/day)	10.94	9.91	11.05	0.34	0.23
% BW	1.76	1.64	1.76	0.07	0.47
Nutrient intake (kg/day)					
OM	9.88	8.96	9.98	0.31	0.23
CP	0.83	0.79	0.82	0.01	0.26
EE	0.18	0.16	0.18	0.01	0.23
NDF	4.97	4.30	5.15	0.24	0.22
ADF	3.15	2.71	3.27	0.16	0.22
Apparent digestibility (%)					
DM	45.78	49.18	48.03	0.58	0.06
OM	49.85	52.72	52.47	0.57	0.06
NDF	37.73	34.26	33.26	2.11	0.66
ADF	19.33	21.50	24.01	1.19	0.20
Estimated energy intake[1]					
MEI (Mcal/day)	18.90	19.17	22.25	0.81	0.18
MEI (Mcal/kg DM)	1.70	1.99	2.03	0.05	0.08
MCP[2] (kg/day)	0.65	0.68	0.76	0.03	0.18

SEM = standard error of the mean, DM = dry matter, OM = organic matter, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber and MEI = metabolize energy intake.

[1]1 kg of digestible organic matter (DOM) = 3.8 Mcal ME (Kearl, 1982)

[2] Microbial crude protein (MCP) = kg DOMI x 0.130 (Kearl, 1982)

Table 4: Effect of vegetable oil on ruminal pH, ammonia–nitrogen, blood urea nitrogen and ruminal volatile fatty acids.

Items	Palm	Soybean	Sunflower	SEM	P-value
Ruminal pH	6.77	6.74	6.70	0.11	0.87
NH_3-N, mg%	10.80	9.45	10.40	1.54	0.83
BUN, mg%	6.89	6.78	5.44	0.71	0.37
Total VFAs, mMol/L	58.77	58.97	59.43	3.32	0.99
VFA, Mol/100 Mol					
Acetic (C_2)	73.61	72.22	72.27	1.31	0.72
Propionic (C_3)	18.23	21.70	20.86	1.16	0.20
Butyric (C_4)	8.16	6.08	6.88	0.69	0.22
C2/C3ratio	4.07	3.33	3.55	0.29	0.29

NH_3-N = ammonia–nitrogen, BUN = blood urea nitrogen, VFA = volatile fatty acid
SEM = standard error of the mean

Table 5: Effect of vegetable oil on DNA copy number of rumen biohydrogenation bacteria (copies/ml).

Items	Palm	Soybean	Sunflower	SEM	P-value
Total Bacteria(10^{12})	1.67	1.62	1.35	0.18	0.22
Bio-hydrogenation bacteria					
Butyrivibrio fibrisolvens (10^6)	1.07	1.10	0.85	0.24	0.52
Butyrivibrio proteoclasticus (10^7)	0.96	1.16	0.92	0.34	0.75
Anaerovibrio lipolytica (10^4)	4.14	2.94	1.66	2.06	0.51
Megasphaera elsdenii (10^3)	1.68	1.59	1.05	0.77	0.69
Ruminococcus albus (10^8)	3.81^b	5.83^a	2.68^b	0.61	<0.01
Cellulolytic bacteria					
Ruminococcus flavefaciens (10^9)	0.93^b	1.46^a	1.33^a	0.15	0.01
Fibrobacter succinogenes (10^6)	2.97	3.09	1.93	1.51	0.71
Prevotella ruminicola (10^6)	1.49^{ab}	3.10^a	0.68^b	0.78	0.03

^{ab} Means in the same row with different superscript differ (P<0.05)
SEM = standard error of the mean

cellulolytic bacteria also depended on fiber in the diet, high level of fiber in diet stimulates activity and growth of cellulolytic bacteria. Kong *et al*., (2010) reported that high fiber diet increased rumen fibrolytic bacteria (*R. albus, R. flavefaciens* and *B. Fibrisolvens*), and a nonfibrolytic bacteria (*P. Ruminicola*). However, the DNA copy numbers of bacteria were also reduced with oil enriched PUFA supplements, indicating that PUFA inhibited their growth. The sensitivity of biohydrogenation bacteria and other bacterial strains to PUFA supplements has been found to be inûuenced by the fatty acid composition (Potu *et al*., 2011). The reduced number of biohydrogenation bacteria and cellulolytic bacteria in SFO treatment could be explained by two effects. First of all, Komgmun *et al*. (2011) reported that vegetable oil may coat rumen digesta and protect digesta from bacterial attachment; consequently, bacteria had an insufficient substrate for growth. The second, in which lipids with high proportions of PUFA, PUFA had inhibited the activity and growth of bacteria (Palmquist, 1988). Gudla *et al*. (2012) reported that supplementation of a blend of fish oil and SBO reduced the DNA abundance of *R. ûavefaciens*, *B. ûbrisolvens* and *R. albus*, especially when added to the high forage diet. On the other hand, a combination of long chain fatty acid (stearic acid, oleic acid, linoleic acid and linolenic acid) in the diet increased *F. succinogenes, R. flavefaciens* and *B. Proteoclasticus* (Jing *et al.*, 2018).

CONCLUSION

Addition of different oil sources at 4 %DM in total diet of dairy cows had a non-negative effect on feed intake and rumen fermentation efficiency, while rumen biohydrogenation and cellulolytic bacteria differed depending on an oil source. Dry matter digestibility and organic matter digestibility tended to be higher in cows fed on SBO and SFO than cows fed on PO (P=0.06). Similarly, DNA copy number of biohydrogenation bacteria (*R. albus*) and cellulolytic bacteria (*R. flavefaciens* and *P. ruminicola*) were higher in cows fed on SBO than that in cows fed on PO and SFO respectively (P<0.05). In summary, SBO was a good source of vegetable oil for rumen digestibility and ruminal biohydrogenation bacteria population.

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