

**Research article****Effect of oil and tannin supplementation on intake, milk yield and milk composition of dairy cows**

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Abstract

Supplementation of oils rich in polyunsaturated fatty acids and condensed tannin has been known as a feeding approach to improve healthy fatty acids in ruminant milk, but it can cause an adverse effect on feed intake and animal performance. This study aimed to investigate the effect of feeding oil alone or in combination with grape seed tannin extract (GSTE) on feed intake, milk yield and composition of dairy cows. Sixteen low production dairy cows in mid-lactation fed a basal diet based on agro-industrial by-products were arranged to a completely randomized design for a 6-week duration. Animals were fed basal diet without oil and GSTE inclusion (CON), 2.5% DM soybean oil (SBO), 2.5% DM blend of soybean oil and tuna fish oil at 3:2 w:w (SFO), or SFO plus 0.4% DM GSTE (OCT). The results showed that DM intake was reduced ($P < 0.05$) by 14.4% in OCT relative to CON. Milk yield was not affected by oil and GSTE supplementation, but SFO and OCT strongly depressed milk fat, protein and total solids ($P < 0.001$). In conclusion, in a low production cow diet based on agro-industrial by-products containing high lipid, supplementation of oil and GSTE should be considered in the aspects of feed intake and milk composition.

Keywords: Cows, Milk composition, Milk yield, Oil, Tannin

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INTRODUCTION

In recent years, human life quality has been increasing, but it also faces with many health challenges due to the consumption of unsafety foods. [Joyce et al. \(2009\)](#) concluded that ingestion of diet containing high saturated fatty acids (SFA) could cause cardiovascular diseases. [WHO \(2003\)](#) recommended that people should decrease the consumption of *trans* fatty acids (FA), but increase the consumption of n-3 FA including C18:3n-3 (alpha-linolenic acid, ALA), C20:5n-3 (eicosapentaenoic acid, EPA) and C22:6n-3 (docosahexaenoic acid, DHA) to reduce the incidence of chronic diseases. Conjugated linoleic acids (CLA), which is mainly synthesized in the rumen, are known to have anti-oxidant, anti-carcinogenic, anti-inflammatory and anti-obesity effects ([Kim et al., 2008](#)). Contents of CLA and n-3 FA in rumen and milk had been improved when ruminant diets were supplied with oilseeds high in linoleic acid (LA) and ALA ([Thanh and Suksombat, 2015](#); [Thanh et al., 2021](#)). Supplementing fish oil and condensed tannin (CT) in soybean oil diet was also a feeding approach to enrich n-3 FA and CLA contents in rumen of goats ([Thanh et al., 2018](#)) and cattle ([Thanh et al., 2022](#)).

In some cases, however, inclusion of oils and CT in dairy cattle diet cause negative effect on intake ([Boerman and Lock, 2014](#)) and animal performance ([Chilliard et al., 2009](#)); thus, the objective of this study was to evaluate the effect of oil added alone or in combination with grape seed tannin extract (GSTE) on feed intake, milk yield and composition of dairy cows raising in Viet Nam fed diet based on agro-industrial by-products containing high lipid.

MATERIALS AND METHODS

Study site

The study conducted in a private dairy cattle farm in Can Tho city, Vietnam. The mean daily temperature and humidity respectively ranged from $28.4 \pm 1.52^{\circ}\text{C}$ and $65.2 \pm 10.4\%$ (at 8:00) to $30.2 \pm 2.05^{\circ}\text{C}$ and $58.2 \pm 6.94\%$ (at 14:00). Samples were analyzed at Laboratory of Ruminant Production Techniques, Department of Animal Sciences, College of Agriculture, Can Tho University.

Animals

Sixteen lactating Holstein cows in mid-lactation averaging 4.42 ± 2.47 months in milk, 447 ± 44.5 kg of live weight, 14.0 ± 3.89 kg of milk/day and $4.00 \pm 0.38\%$ of fat milk content were used in this experiment. They were kept in individual tie stalls, had enough space to walk and free access to water and mineral block. All animals were fed daily rations as equal meals at 05:00 and 12:00. Prior to conduct the experiment, the cows were freely fed a basal diet for 1 week to determine the maximum feed intake. On the last 2 days of the pre-feeding period, milk yield was recorded, milk composition and somatic cells were analyzed.

Experimental design and diets

The cows were assigned to a completely randomized design with four replicates per each treatment. The study lasted for 6 weeks consisting 2 weeks for adjustment, followed by 4 weeks for sampling. A basal diet mainly based on agro-industrial by-products were fed to all animals. Treatments included the basal diet without oil and GSTE supplement as a control (CON), 2.5% soybean oil (SBO), 2.5% blend of soybean oil and tuna oil (3:2, w:w) (SFO), or SFO plus 0.4% GSTE (OCT). Oils and GSTE were daily mixed with commercial concentrate before feeding to the cows. Agro-industrial by-products and elephant grass were then fed to the cows. A pure product of soybean oil was used in this study while tuna oil was added as a crude oil. A commercial grape seed tannin extract was used in the current study as a source of CT, in form of proanthocyanidin (PA). Nutrient requirements of lactating dairy cows proposed by NRC (2001) were used to formulate all diets in this study (Table 1).

Table 1 Feed ingredients of diets

Ingredient (% DM)	Diet ¹			
	CON	SBO	SFO	OCT
Concentrate	22.0	18.4	18.4	17.9
Soybean waste	7.00	7.10	7.10	7.40
Wet brewers' grains	11.0	13.5	13.5	13.5
Pineapple peel silage	20.0	20.0	20.0	20.0
Jackfruit by-product	30.0	28.5	28.5	28.3
Elephant grass	10.0	10.0	10.0	10.0
Soybean oil	-	2.50	1.50	1.50
Tuna oil	-	-	1.00	1.00
Grape seed tannin extract	-	-	-	0.40
Total	100	100	100	100
Concentrate + CP-supplemented by-products ²	40.0	39.0	39.0	38.8
Forage ³	60.0	58.5	58.5	58.3
Supplement ⁴	-	2.50	2.50	2.90

¹CON: control, SBO: 2.5% soybean oil, SFO: 2.5% soybean oil and tuna oil (3:2, w:w), OCT: 2.5% soybean oil and tuna oil (3:2, w:w) + 0.4% grape seed tannin extract. ²concentrate + soybean waste + wet brewers' grains.

³pineapple peel silage + jackfruit (by-product) + elephant grass. ⁴soybean oil + tuna oil + grape seed tannin extract

A commercial concentrate for lactating cows was used in this study, whereas agro-industrial by-products was readily available with large amount in Can Tho city. Soybean oil was supplemented as a pure product while crude tuna fish oil was used. GSTE (IBPharco Co., Ltd., Ha Noi, Viet Nam) was supplied as a commercial source of CT, which contained 95% proanthocyanidins. Table 2 presents the chemical compositions of the individual feeds and experimental diets using in this study. Concentrate, soybean waste and wet brewers' grains, which contained 17.4, 20.4 and 26.5% CP, respectively, were the main sources of protein. High contents of NDF were found in elephant grass (62.3%) and pineapple peel silage (55.1%). Lipid content was quite high in some feeds such as soybean waste (8.24%), jackfruit (by-product) (6.63%) and wet brewers' grains (5.94%). The CON diet had 4.75% lipid content, whereas these were 7.16-7.17% in oil and GSTE added diets.

Table 2 Chemical composition of feeds and diets

Item	Chemical composition (% DM) ¹								
	DM	OM	Ash	CP	EE	NDF	ADF	NFC	NE _L ²
Feed									
Concentrate	89.9	93.6	6.44	17.4	3.74	28.0	13.5	44.4	1.61
Soybean waste	11.1	96.2	3.75	20.4	8.24	23.4	16.7	44.3	1.83
Wet brewers' grains	22.9	96.6	3.43	26.5	5.94	49.9	20.7	14.3	1.41
Pineapple peel silage	10.4	93.2	6.84	6.82	2.55	55.1	29.6	28.7	1.24
Jackfruit (by-product)	20.7	93.5	6.47	10.3	6.63	24.6	19.4	52.1	1.50
Elephant grass	16.0	88.8	11.2	8.23	1.97	62.3	39.9	16.3	1.11
Soybean oil	100	100	-	-	100	-	-	-	4.95
Tuna oil	100	100	-	-	100	-	-	-	4.95
Grape seed tannin extract	100	-	-	-	-	-	-	-	-
Diet ³									
CON	32.9	93.5	6.49	13.4	4.75	37.9	22.2	37.4	1.45
SBO	32.5	93.8	6.25	13.3	7.17	37.8	21.9	35.4	1.53
SFO	32.5	93.8	6.25	13.3	7.17	37.8	21.9	35.4	1.53
OCT	32.4	93.4	6.21	13.3	7.16	37.7	21.9	35.2	1.52

¹DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, CF: crude fiber, NFC: non-fiber carbohydrate. ²NEL: net energy for lactation (Mcal/kg DM).

³CON: control, SBO: 2.5% soybean oil, SFO: 2.5% soybean oil and tuna oil (3:2 w:w), OCT: 2.5% soybean oil and tuna oil (3:2 w:w) + 0.4% grape seed tannin extract.

Sampling, measurements and chemical analysis

During the collection period, feeds offered and the residuals were recorded daily, and feed samples were collected for two consecutive days weekly to calculate daily feed intake. Feed samples were collected and dried (FD 53, Binder, Germany) at 60°C for 72 h.

At the end of the experimental period, samples of feed and oils were pooled and representative samples were used for further chemical analysis. Samples were ground (Cutting Mill SM100, Retsch, Germany) through a 1-mm screen and used for proximate analysis including dry matter (DM), crude protein (CP), total minerals (Ash), ether extract (EE) and crude fiber (CF) following the standard methods of [AOAC \(1990\)](#). Neutral detergent fiber and acid detergent fiber (ADF) were determined using the method described by [Van Soest et al. \(1991\)](#). Total digested nutrients (TDN) of feeds and oils were calculated following the equations of [Jayanegara et al. \(2019\)](#), whereas net energy for lactation (NE_L; Mcal/kg DM) of feeds and oils was calculated following the equations of [NRC \(2001\)](#). All chemical components were expressed on DM basis.

The dairy cows were milked daily and milk yields were recorded at each milking time (5:00 and 14:00 h). Milk samples were collected weekly in 2 consecutive milking days and analyzed for milk composition including fat, protein, lactose, solid not fat and total solids using a MilkoScan Mars infrared automatic analyzer (Foss, Hillerød, Denmark). Somatic cell counts in milk samples were measured at the start and end of the experiment using a milk somatic cell analyzer (Adam-SCC, Nano Entek Inc, Korea).

Statistical analysis

Data on somatic cell counts were analyzed by ANOVA procedure of SAS University Edition 2019 (SAS Institute Inc, NC, USA). Other data were analyzed according to a completely randomized design with the repeated

measures (weeks) using PROC MIXED procedure of SAS. Significant differences among treatment means were assessed by Tukey's multiple comparison tests. Differences were declared significant at $P < 0.05$, and the tendency was declared at $0.05 \leq P < 0.1$.

RESULTS

Intake

Treatment diets had affected feed and nutrient intakes (Table 3). Intake of DM was highest in the CON with 10.7 kg/day and lowest in OCT with 9.16 kg/day ($P < 0.05$). Feeding combination of oil blend and GSTE reduced DM intake leading to decrease intakes of all nutrients ($P < 0.05$). While DM intake remained unchanged in blend oil diet, a reduction of DM intake in blend oil and GSTE diet suggested that CT from GSTE was the main reason to reduce feed ingestion in experimental cows.

Table 3 Feed and nutrient intakes

Intake (kg DM/day)	Diet ¹				SEM	P-value		
	CON	SBO	SFO	OCT		Diet (D)	Time (T)	D×T
DM	10.7 ^a	10.2 ^{ab}	10.3 ^{ab}	9.16 ^b	0.32	0.011	0.244	1.000
OM	9.94 ^a	9.57 ^{ab}	9.65 ^{ab}	8.53 ^b	0.30	0.009	0.223	1.000
CP	1.44 ^a	1.37 ^{ab}	1.38 ^{ab}	1.23 ^b	0.04	0.005	0.137	0.999
EE	0.53 ^c	0.77 ^a	0.78 ^a	0.70 ^b	0.02	<0.001	0.019	0.969
NDF	4.15 ^a	3.97 ^{ab}	3.98 ^{ab}	3.52 ^b	0.13	0.011	0.002	1.000
ADF	2.33 ^a	2.23 ^{ab}	2.23 ^{ab}	1.97 ^b	0.08	0.013	0.062	1.000
NE _L , Mcal/day	15.3 ^{ab}	15.5 ^{ab}	15.7 ^a	13.8 ^b	0.47	0.030	0.214	0.999

DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, NE_L: net energy for lactation. ¹CON: control, SBO: 2.5% soybean oil, SFO: 2.5% soybean oil and tuna oil (3:2 w:w), OCT: 2.5% soybean oil and tuna oil (3:2 w:w) + 0.4% grape seed tannin extract. ^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

Milk yield and composition

Milk yield ranged from 11.5 to 13.3 kg/day and was not affected by diets in this study (Table 4). Compared with the control, milk fat content was reduced ($P < 0.001$) by 20.5 and 15.7% in SFO and OCT diets, respectively. Moreover, feeding SFO and OCT diets led to reduce ($P < 0.01$) milk protein and total solid, whereas no effect was observed when cows received SBO diet relative to control cows. The lowest milk fat concentration was detected in fish oil-contained diets (3.45-3.48%) at 2-week feeding ($P < 0.05$; Figure 1A). Compared with CON cows, SFO cows showed the lower milk fat concentration, particularly at 5 and 6 weeks of feeding ($P < 0.05$; Figure 1A). Cows fed OCT resulted in a reduction of milk protein concentration at 4-week feeding ($P < 0.05$; Figure 1B) and milk solid not fat ($P < 0.05$; Figure 1C) from 3 to 5 weeks of feeding compared to those in SBO cows. Compared with OCT cows (11.3-11.5%), milk total solid was higher in CON cows (12.6-12.7%) during the last two weeks of feeding ($P < 0.05$; Figure 1D). Somatic cell counts in milk were not affected by diets in this study.

Table 2 Milk yield and composition of experimental cows

Item	Diet ¹				SEM	P-value		
	CON	SBO	SFO	OCT		Diet (D)	Time (T)	D×T
Milk yield								
Kg milk/day	13.6	13.0	14.0	12.0	0.61	0.111	0.176	1.000
Kg milk/kg DMI	1.28	1.26	1.39	1.28	0.05	0.273	0.827	1.000
Composition, %								
Fat	4.15 ^a	3.83 ^{ab}	3.30 ^c	3.50 ^{bc}	0.10	<0.001	<0.001	0.406
Protein	3.25 ^{ab}	3.37 ^a	3.20 ^b	3.14 ^b	0.04	0.001	0.624	0.998
Lactose	4.56 ^a	4.67 ^a	4.62 ^a	4.35 ^b	0.04	<0.001	0.238	1.000
Solid not fat	8.65 ^b	8.96 ^a	8.69 ^{ab}	8.21 ^c	0.07	<0.001	0.922	1.000
Total solid	12.6 ^a	12.6 ^a	11.8 ^b	11.5 ^b	0.13	<0.001	0.028	0.930
Somatic cell count, ×10 ³ /mL								
Initial	2,560	1,254	1,217	929	458	0.107	-	-
Final	1,862	1,781	1,187	1,012	450	0.475	-	-
Difference	-698	527	-30.2	82.8	605	0.570	-	-

DMI: dry matter intake. ¹CON: control, SBO: 2.5% soybean oil, SFO: 2.5% soybean oil and tuna oil (3:2 w:w), OCT: 2.5% soybean oil and tuna oil (3:2 w:w) + 0.4% grape seed tannin extract. ^{a-c}Means within a row with different superscripts are significantly different (P < 0.05).

DISCUSSION

Intake

That reduced DMI in tannin-containing diet in this study was supported by [Focant et al. \(2019\)](#), who reported that dietary inclusion of 0.87% oak tannin decreased DM and OM intakes in dairy cows. [Aguerre et al. \(2016\)](#) showed that supplementation of tannin extract from Quebracho decreased CP digestibility in dairy cows, and these authors also observed a negative correlation between CP digestibility and Quebracho tannin extract rate in the diet. Similar result was also observed when heifers were infused Quebracho tannin extract via rumen. The absorption of nutrients from the small intestine can be decreased when tannin is added ([McNabb et al., 1998](#); [Silanikove et al., 2001](#)). For this reason, [Yanza et al. \(2021\)](#) showed that tannin can cause the negative effects on digestibility in ruminants by coating the physical forms of feed particles due to the complex binding of tannin–protein or tannin–fiber.

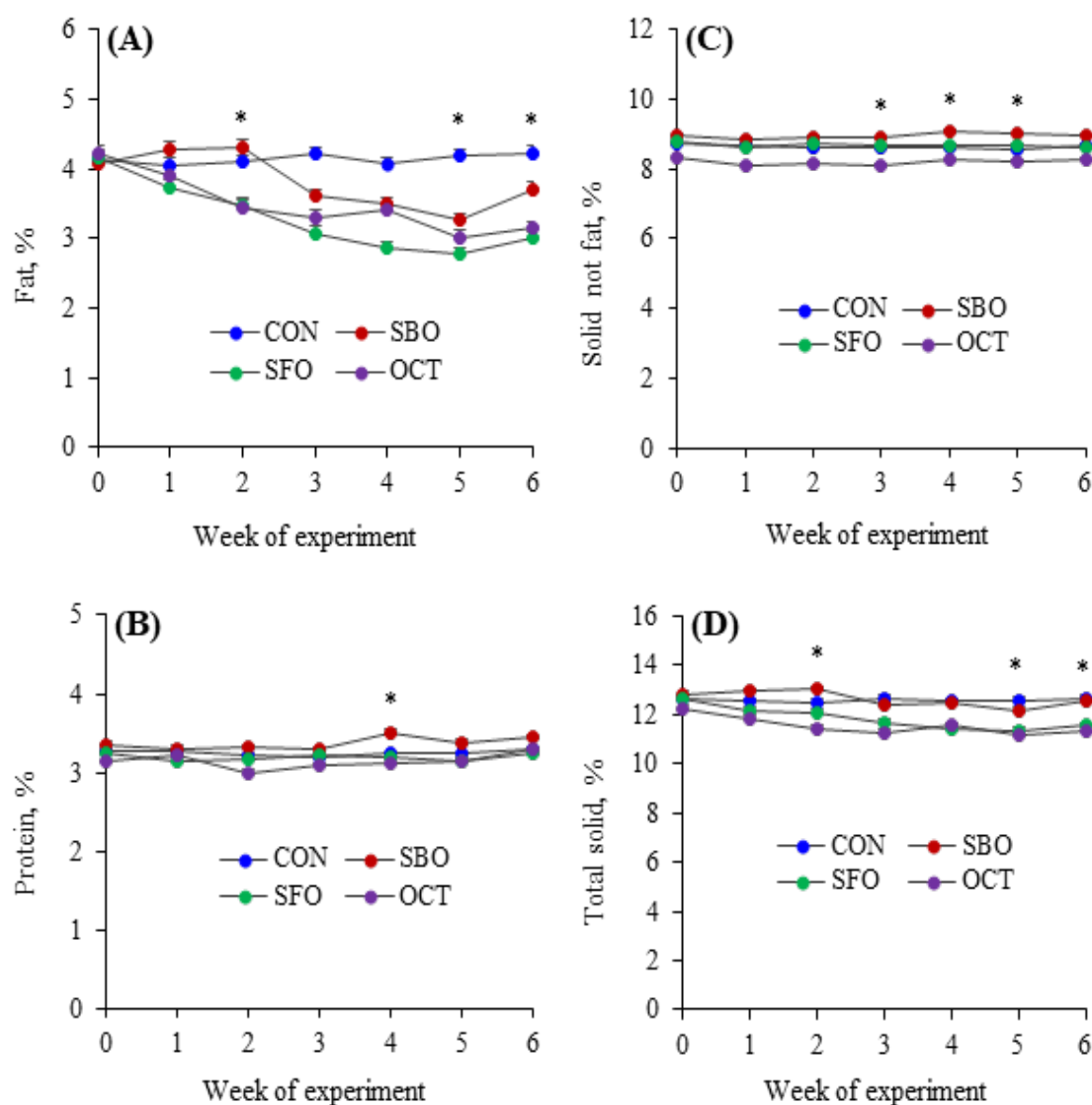


Figure 1 Milk composition changes of treatment groups during entire experiment. * $P < 0.05$.

Milk yield and composition

Milk production of cows in this study was lower than cows raised in an intensive farm (14.2 kg milk/day), which reported by [Thanh et al. \(2021\)](#). Cows fed soybean oil were reported to have greater milk production and fat content as compared with those fed fish oil ([Ramaswamy et al., 2001](#); [Whitlock et al., 2002](#); [Fatahnia et al., 2008](#)). It was proved with the finding of this study. However, [Ha et al. \(2023\)](#) found no effect of oil and grape seed tannin extract on milk yield and milk fat of dairy goats. [Szczechowiak et al. \(2016\)](#) found that feeding combination of oil blend from soybean oil plus fish oil and plant-extracted tannins had no effect on milk yield and composition of dairy cows. This may be due to the lower production cows and higher fat content in basal diet of this study compared with experiment of [Szczechowiak et al. \(2016\)](#). That milk fat depression in cows fed diets containing fish oil was in agreement with the findings of [Thanh et al. \(2023\)](#), who reported a reduction of milk fat when cows received 3% of linseed oil, sunflower oil, and tuna crude oil in their diets. Supplementing oils rich in polyunsaturated fatty acids typically disturbs ruminal

fermentation and fiber digestibility, resulting in reduced acetate production and milk fat synthesis. (Coppock and Wilks, 1991). Moreover, high-oil diets rich in polyunsaturated fatty acids increased *trans*-10,*cis*-12 conjugated linoleic acid, resulting in a decrease in the expression of genes involved in lipid synthesis in the mammary gland (Harvatine and Bauman, 2006). Thanh et al. (2023) also detected an increase in milk *trans*-10,*cis*-12 conjugated linoleic acid when cows were fed 3% added oil.

CONCLUSION

Supplementation of 2.5% soybean oil in the diet has no effect on feed intake, milk yield and composition of cows; however, marked reduction of these parameters were observed when cows were fed 2.5% blend oil from soybean oil plus tuna oil in combination with or without 0.4% grape seed tannin extract. Thus, low production cow diet based on agro-industrial by-products containing high lipid should use less than 2.5% additional fat.

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AUTHOR CONTRIBUTIONS

Lam Phuoc Thanh, Tran Thi Thuy Hang; Conceptualization and design the experiment, investigation, supervision, editing and finalization

Pham Truong Thoai Kha, Nguyen Thi Thu Ha, Duong Tran Tuyet Mai, Mai Hoan Tu; Investigation, methodology, formal analysis, manuscript preparation

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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