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Effects of full fat oil seeds on milking performance, milk composition and milk quality in lactating Holstein cows

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ABSTRACT. This study was conducted to investigate the effect of full-fat oil seeds on milking performance, milk composition, and milk quality in lactating Holstein cows. Animals for the experiment were randomly assigned to one of three treatment groups. The control group was fed a CON diet (total mixed ration + hay + concentrate), T1 was fed the CON diet + 600 g flaxseed, and T2 was fed the CON diet + 600 g flaxseed + 200 g chia seeds. Supplementation with flaxseed or flaxseed + chia seeds had little effect on milk yield, feed intake, and feed efficiency of lactating Holstein cows. The content of protein and non-fat-solids was higher in T1 than in control ($P < 0.05$) during the first period, but overall, the addition of flaxseed or flaxseed + chia seeds had a low influence on milk composition. Conjugated linoleic acid (CLA) and polyunsaturated fatty acid contents increased with the supplementation of flaxseed or flaxseed + chia seeds ($P < 0.05$). The percentage of n-3 fatty acids and the antioxidant activity of raw milk also increased as a result of flaxseed or flaxseed + chia seed administration ($P < 0.05$). Thus, in this study, mixed flaxseed and chia seed supplementation exerted a positive effect on lactation persistency, functional fatty acid concentration (CLA, polyunsaturated fatty acid, and n-3 fatty acid), and antioxidant activity in lactating Holstein cows.

Introduction

The Ministry of Agriculture, Food and Rural Affairs (MAFRA), 2020 has reported that consumption of dairy products in Korea is increasing every year, and the demand for functional milk and dairy products with additional health benefits is also steadily growing. Recently, attempts have been made to enhance the functional ingredients of milk, including constantly increasing interest in polyunsaturated fatty acids (PUFAs) due to their effectiveness in the prevention of cancer, hypertension, and cardiovascular diseases (Ander et al., 2003). Among fatty acids contained in food, n-3 fatty acids have been reported to particularly contribute to reduced incidence of cardiovascular and cerebrovascular diseases (Stewart et al., 2001). In addition, there has also been an attempt to increase the conjugated linoleic acid (CLA) content in meat and milk due to their anticancer properties (Ip et al., 1994). PUFAs can be found in foods such as fish and vegetable fats, and are known to be abundant in full-fat oil seeds. Typically, the fat content of flaxseed (*Linum usitatissimum* L.) is approximately 40%, of which about 55% is α-linolenic acid (Petit, 2002). Meanwhile, the fat content of chia seeds (*Salvia hispanica* L.) is about 25–38%, and its α-linolenic acid (approx. 60%) and protein (19–23%) contents are known to be higher compared to grains such as wheat, corn, oat, and barley (Ayerza, 1995). In general, milk has a high saturated fatty acid content and low n-3 fatty

acid content (Kennelly, 1996). However, it has been reported that feeding vegetable oil (Bu et al., 2007), marine oil (Rego et al., 2005), and algae (Franklin et al., 1999) to lactating cows can alter the fatty acid composition of their milk. Previous studies have mainly focused on changes in the fatty acid composition of milk as a result of flaxseed or chia seed addition to the diet of lactating cows, while research on milk production and quality is lacking. Therefore, this study was conducted to examine the effect of flaxseed and flaxseed + chia seed supplementation on milk yield, milk composition, fatty acid composition, and milk quality in lactating Holstein cows.

Material and methods

This study followed all animal experimental procedures, as indicated by the Kangwon National University Animal Experimental Ethics Committee (Institutional Animal Care and Use Commitee, IACUC).

Animals, treatments, and management

This study was conducted using 15 early lactating Holstein cows (milk yield: 36.2 kg, parity: 2.5) over a period of 135 days. Fifteen early lactating Holstein cows were randomly assigned to one of three dietary treatment groups based on a completely randomized block design. The control group was fed the CON diet (total mixed ration (TMR) + commercial concentrate + hay), the T1 group was fed the CON diet + 800 g (cow/day) of flaxseed, while the T2 group was fed the CON diet + 600 g (cow/day) flaxseed + 200 g chia seed.

The cows were fed a total mixed ration, commercial concentrate, and hay (timothy + tall fescue) twice daily (08:00 and 17:00). The supply of each nutrient in relation to the cows' requirements was calculated for different lactation stages based on the guidelines of the National Research Council (NRC, 2001). Flaxseed and chia seeds were mixed and fed to the cows throughout the experimental period as they consumed the concentrate. TMR, basal concentrate feed and the amount of hay were constant across treatment groups, but early lactating cows and high-yielding cows were given an additional 2 kg of high energy concentrate for every 5 kg of increased milk yield to meet the nutrient demand compared to average milk yield. Water was always freely available and other feeding management activities were conducted in accordance with farm practices. The chemical composition of the experimental diets are presented in Table 1, and the fatty acid composition of flaxseed and chia seeds are listed in Table 2.

Table 1. Ingredients and chemical composition of experimental diets

Item	Total mixed ration	Concentrate I	Concentrate II
Ingredient composition, %			
Concentrated feed	40.00	–	–
Corn grain	3.80	12.97	24.79
Wheat grain	–	12.42	15.00
Cane molasses	5.00	3.00	3.00
Wheat flour	–	2.00	20.00
Wheat bran	–	5.00	–
Corn gluten feed	7.00	21.00	11.44
DDGS (corn)	–	4.00	4.00
Soybean meal	–	15.80	12.00
Coconut meal	–	6.00	0.00
Palm kernel meal	–	8.00	4.00
Sunflower seed meal	–	5.00	–
Whole cottonseed	8.00	–	–
Beet pulp	5.00	–	–
Alfalfa hay	8.00	–	–
Timothy hay	17.00	–	–
Klein grass hay	3.00	–	–
Tall fescue straw	3.00	–	–
Ca salts of fatty acids	–	0.70	4.12
Salt dehydrated	–	0.80	0.50
Limestone	–	2.19	0.48
Sodium bicarbonate	–	0.40	–
Vitamin premix[1]	0.10	0.20	0.20
Mineral premix[2]	0.10	0.20	0.20
Feed additives	–	0.32	0.27
Chemical composition (as-basis %)			
dry matter	86.92	88.85	88.31
crude protein	15.00	20.53	15.00
ether extract	3.84	5.27	8.34
crude fiber	15.16	7.00	3.83
NDF	34.89	24.28	14.90
ADF	19.90	12.92	7.54
crude ash	6.32	7.21	3.66
Ca	0.63	1.00	0.90
P	0.42	0.52	0.36
TDN	64.01	75.95	86.65
NE_L	1.44	1.64	1.89

Concentrate I – basal diet for every lactating cows; Concentrate II – high energy diet for high producing cows; [1] vitamin premix provided the following quantities of vitamins per kilogram of the diet: IU: vitamin A 10 000, vitamin D_3 1 500, vitamin E 25; [2] mineral premix provided the following quantities of minerals per kilogram of the diet: mg: Fe 50, Cu 7, Zn 30, Mn 24, I 0.6, Co 0.15, Se 0.15; NDF – neutral detergent fiber, ADF – acid detergent fiber, TDN – total digestible nutrients (calculated value), NE_L – net energy for lactation (calculated value)

Milk performance

Milk yield was measured three times during the experimental period using a portable milk meter (EZ-Test, Itec, Tel Aviv-Yafo, Israel). Milk yield was examined twice a day (7:00 and 19:00) for three consecutive days, and the average daily production was calculated by summing the amount of milk pro

Table 2. Fatty acid composition of flax and chia seeds

Item	Flaxseed	Chia seed
Palmitic (C16:0), %	4.99	6.13
Stearic (C18:0), %	3.74	3.01
Oleic (C18:1), %	17.72	6.28
Linoleic (C18:2n-6), %	16.06	18.75
Linolenic (C18:3n-3), %	51.29	59.53

duced in the morning and afternoon. Feed intake was checked daily in pen units, and calculated from the difference between amount of feed and residue. Feed efficiency was calculated using milk yield and dry matter intake.

Milk composition

For the measurement of milk composition, when measuring milk yield, the sample was placed in a 50 ml sample bottle, and 1 g of potassium dichromate was added before storage in the refrigerator. Milk fat, milk protein, solids-not-fat (SNF), milk urea nitrogen (MUN), and somatic cell count (SCC) were subsequently analysed using an automatic milk component analyser (Automatic IR 4000/5000 Milk Analyzer, Foss, Hilleroed, Denmark).

For fatty acid analysis, milk fat was extracted according to the ISO 14156 protocol. Twenty millilitres of milk, 16 ml of ethanol, and 4 ml of ammonia water were mixed in a separatory funnel. To this mixture, 20 ml of diethyl ether was added, stirred, and allowed for layer separation. After separation, 20 ml of pentane was added and stirred, and the liquid from the lower layer was removed. Subsequently, 20 ml of 10% (w/v) sodium sulphate solution was added and gently mixed to remove the lower layer. Then, 20 ml of sodium sulphate solution was added and stirred vigorously for 1 min. After the layers clearly separated, the lower layer was removed and the remaining layer was transferred to an Erlenmeyer flask to which 2 g of sodium sulphate was added. After incubation for 10 min, the solvent was filtered and evaporated in a vacuum concentrator at 50 °C. The remaining solvent was dried for 1 min using nitrogen gas and subsequently converted into fatty acid methyl esters (FAME). A 100 mg sample was weighed in a test tube, mixed with 5 ml of pentane, and 0.2 ml of transesterification reagent was added. This mixture was then stirred vigorously for 1 min, and allowed to react for 5 min. A weighed amount of 0.5 g sodium hydrogen sulphate was added, and the mixture was stirred again and centrifuged at room temperature (350 *g*, 3 min). The resulting supernatant was used for analysis. Fatty acid measurements were conducted using a gas chromatograph (Agilent 6890N, Agilent Technologies, Santa Cara, CA, USA) with an HP-INNOWax column (30 m × 0.32 mm × 0.25 μm, Agilent, Santa Cara, CA, USA).

Volatile compound contents, antioxidant activity, and total cell count

A weighted amount (20 g) of raw milk was placed in a headspace vial (50 ml), sealed with a Teflon-coated septa, and pre-warmed in a water bath at 50 °C for 30 minutes. The pre-warmed sample was then extracted for 30 min at 50 °C using SPME (solid-phase microextraction). The measurement of total volatile fragrance components was carried out using an ACME 6000 gas chromatograph (Young Lin Co., Seoul, Republic of Korea) with an HP-FFAP column (30 m × 0.32 mm ID × 0.25 μm film thickness, Agilent Technologies, Santa Cara, CA,USA).

To measure antioxidant activity, freeze-dried milk powder was dissolved in potassium phosphate buffer (100 mM, pH 7.4) to prepare sample solution. A stock solution of 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was then prepared by adding 88 μl of 140 mM potassium persulfate ($K_2S_2O_8$) to 5 ml of 7 mM ABTS, and incubated for 16 h in the dark. Potassium phosphate buffer (0.1 M, pH 7.4) was used to prepare ABTS radical working solution (absorbance of 0.70 ± 0.02 at 734 nm). To measure ABTS radical scavenging activity, 100 μl of sample solution and 1900 μl of ABTS working solution were mixed and allow to react for 10 min at room temperature before measuring the absorbance at 734 nm. Total antioxidant activity was subsequently calculated using the following formula:

Antioxidant activity (%) = (Control OD-Sample OD) / Control OD × 100.

For microbial testing, 1 ml of raw milk was dispensed into 9 ml of 0.1% peptone solution and diluted by a decimal dilution method. One millilitre of the diluted solution was dispensed into a Petri dish, followed by pouring tryptic soy agar and incubation at 37 °C for 37 h. After this time, the cultured communities were counted and expressed as log number (CFU/ml).

Thiobarbituric acid reaction and oxygen content

To measure the thiobarbituric acid reaction, 2 ml of raw milk was transferred to a centrifuge tube and heated to 40 °C. An aliquot (2 ml) of 5% trichloroacetic acid solution and 7.2% butylated hydroxyl toluene solution were subsequently added and stirred. To this mixture, 2 ml of 1% thiobarbituric acid solution was added and allowed to react in

a water bath at 70 °C for 1 h. Upon completion of the reaction, the sample was centrifuged at 2700 *g* for 5 min, the supernatant was collected, and absorbance was measured at 526 nm using a spectrophotometer (Spectronics 21D, B&L, New York, NY, USA). Subsequently, the malondialdehyde (MDA) content of the sample was quantified and expressed as mg MDA per kg.

To measure the oxygen content, 10 g of raw milk was transferred to a 50 ml serum bottle, completely sealed with an aluminium cap and Teflon-coated septa. Sterilized and non-sterilized samples were prepared separately at 70 °C for 10 min and stored in a cold room at 10 °C and 2 200 lux illumination. Oxygen concentrations were determined on days 0, 1, 3, and 5 by collecting 200 µl of headspace gas with a gas tight syringe (SGE Gas Tight Luer Lock Syringe, Supelco, Saint Louis, MO, USA) and subjecting it to gas chromatography using an ACME 6000 gas chromatography system (Young Lin Co., Seoul, Republic of Korea) equipped with a thermal conductivity detector and a 60/80 Molecular Sieve 13X (1.8 m × 26 mm, Supelco, Saint Louis, MO, USA).

Statistical analysis

All data were analysed using the GLM procedure in the SAS package 9.1 software (SAS Institute Inc., Cary, NC, USA). Significant differences between the treatments were analysed using Tukey's test at a 95% significance level (SAS, 1999).

Results

Table 3 shows the effect of full-fat oil seed supplementation on milk production, feed intake, and feed efficiency in early lactating Holstein cows. During the first lactation period (days 0 to 67), milk yield was higher in the control group than in the T1 and T2 groups ($P < 0.05$). However, in the second lactation period (68 to 135 days), milk yield was similar in all treatment groups due to the rapid decrease in milk yield in the control group. Consequently, there was no difference in milk yield over the entire period between the treatment groups. Moreover, supplementation with flaxseed or flaxseed + chia seeds had little effect on feed intake and feed efficiency.

Table 4 shows the effect of the addition of full-fat oil seeds on milk composition. During the first period, milk protein and SNF were higher in the T1 group compared to the control group ($P < 0.05$), while milk lactose levels were the highest in the T2 group ($P < 0.05$). The milk fat content showed an increasing tendency in the T2 group, but there was no statistically significant difference in these values.

Table 3. Effect of full fat oil seeds on milk yield and feed intake of Holstein cows

Item	CON	T1	T2
Milk yield, kg/day			
1st period	35.73 ± 9.22^{a}	32.11 ± 7.30^{b}	33.24 ± 4.43^{ab}
2nd period	32.39 ± 8.34	29.53 ± 4.00	32.53 ± 6.00
whole period	33.63 ± 9.32	30.58 ± 5.58	32.85 ± 5.23
Feed intake, dry matter kg/cow/day			
concentrate	12.27 ± 0.07	10.12 ± 0.05	10.84 ± 0.06
hay (timothy and tall fescue)	7.67 ± 0.03	7.21 ± 0.04	7.12 ± 0.03
total mixed ration	7.44 ± 0.05	7.09 ± 0.05	6.91 ± 0.04
flaxseed	–	0.80	0.20
chia seed	–	–	0.60
DMI	27.38 ± 0.11	25.22 ± 0.08	25.67 ± 0.09
Feed efficiency, milk yield/DMI, kg/kg	1.23 ± 0.03	1.21 ± 0.02	1.28 ± 0.03

CON – control diet (concentrate + total mixed ration + hay), T1 – CON diet + 800 g of flaxseed, T2 – CON diet + 600 g of flaxseed + 200 g of chia seed; 1st period – first lactation (0 to 67 days), 2nd period – second lactation (68 to 135 days); DMI – dry matter intake; data are presented as mean value ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

Table 4. Effect of full fat oil seeds on milk composition of Holstein cows

Item		CON	T1	T2
1st period	Protein, %	2.98 ± 0.22^{b}	3.28 ± 0.28^{a}	3.06 ± 0.16^{a}
	Fat, %	3.49 ± 0.23	3.72 ± 0.46	3.44 ± 0.25
	Lactose, %	4.74 ± 0.15^{ab}	4.68 ± 0.12^{b}	4.84 ± 0.15^{a}
	SNF, %	8.47 ± 0.18^{b}	8.68 ± 0.20^{a}	8.63 ± 0.08^{ab}
	MUN, mg/dl	11.29 ± 0.68	11.41 ± 1.14	11.91 ± 0.65
	SCC, 10^3/ml	76.00 ± 64.08	99.64 ± 38.25	79.68 ± 61.88
2nd period	Protein, %	3.22 ± 0.21	3.42 ± 0.38	3.15 ± 0.14
	Fat, %	3.57 ± 0.35	3.78 ± 0.58	3.69 ± 0.39
	Lactose, %	4.76 ± 0.12^{ab}	4.64 ± 0.17^{b}	4.88 ± 0.11^{a}
	SNF, %	8.76 ± 0.26	8.84 ± 0.48	8.85 ± 0.08
	MUN, mg/dl	12.52 ± 2.18	11.90 ± 1.53	12.69 ± 0.92
	SCC, 10^3/ml	98.38 ± 38.06	129.71 ± 77.82	106.50 ± 1.83

CON – control diet (concentrate + total mixed ration + hay), T1 – CON diet + 800 g of flaxseed, T2 – CON diet + 600 g of flaxseed + 200 g of chia seed; 1st period – first lactation (0 to 67 days), 2nd period – second lactation (68 to 135 days); SNF – solids-not-fat, MUN – milk urea nitrogen, SCC – somatic cell count; data are presented as mean value ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

In addition, MUN and SCC were similar between the treatment groups. Milk lactose levels in the second period had increased in the T2 group ($P < 0.05$), similar to the first period, but there were no differences between treatment groups with respect to milk protein, fat, SNF, MUN, and SCC.

Table 5 shows the effect of the supplementation of full-fat oil seed on the fatty acid composition of raw milk. Although there was a small effect of the supplementation of flaxseed and flaxseed + chia

Table 5. Effect of full fat oil seeds on fatty acid composition in milk of Holstein cows

Item	CON	T1	T2
Butyric acid (C4), %	2.437 ± 0.121	2.451 ± 0.026	2.514 ± 0.011
Caproic acid (C6:0), %	1.839 ± 0.066	1.923 ± 0.026	1.922 ± 0.015
Caprylic acid (C8:0), %	1.251 ± 0.022	1.351 ± 0.017	1.321 ± 0.018
Capric acid (C10:0), %	3.076 ± 0.038	3.352 ± 0.039	3.281 ± 0.042
Lauric acid (C12:0), %	4.820 ± 0.028	5.035 ± 0.037	4.982 ± 0.058
Myristic acid (C14:0), %	13.946 ± 0.105	13.393 ± 0.026	14.207 ± 0.027
Pentadecanoic acid (C15:0), %	1.067 ± 0.016	1.056 ± 0.009	1.011 ± 0.008
Palmitic acid (C16:0), %	33.913 ± 0.400	32.727 ± 0.265	33.030 ± 0.069
Heptadecanoic acid (C17:0), %	0.468 ± 0.012	0.490 ± 0.003	0.484 ± 0.012
Stearic acid (C18:0), %	9.940 ± 0.682	10.963 ± 0.089	10.692 ± 0.281
SFA, %	72.756 ± 1.490	72.741 ± 0.538	73.444 ± 0.542
Dodecenoic acid (C12:1), %	0.167 ± 0.006	0.164 ± 0.001	0.152 ± 0.003
Myristoleic acid (14:1), %	1.199 ± 0.079	1.140 ± 0.014	1.110 ± 0.035
Palmitoleic acid (16:1n7), %	1.712 ± 0.115	1.345 ± 0.003	1.261 ± 0.033
Oleic acid (18:1n9), %	20.408 ± 0.031	20.764 ± 0.264	19.994 ± 0.137
Heneicosenoic acid (21:1), %	0.047 ± 0.003	0.046 ± 0.001	0.049 ± 0.004
MUFA, %	23.534 ± 0.234	23.460 ± 0.282	22.567 ± 0.211
Linoleic acid (18:2n6), %	2.453 ± 0.067	2.494 ± 0.051	2.604 ± 0.021
Gamma linoleic acid (18:3n6), %	0.111 ± 0.004	0.106 ± 0.003	0.110 ± 0.001
Alpha linoleic acid (18:3n3), %	0.208 ± 0.005	0.216 ± 0.009	0.237 ± 0.010
CLA, %	0.474 ± 0.016^{c}	0.492 ± 0.006^{b}	0.545 ± 0.003^{a}
Eicosatrienoic acid (20:3), %	0.190 ± 0.022	0.214 ± 0.004	0.210 ± 0.054
Arachidonic acid (20:4n6), %	0.185 ± 0.012	0.179 ± 0.002	0.180 ± 0.003
Eicosapentaenoic acid (20:5n-3), %	0.050 ± 0.003	0.057 ± 0.002	0.061 ± 0.004
Docosapentaenoic acid (22:4n6), %	0.039 ± 0.001	0.040 ± 0.001	0.043 ± 0.003
Total n3, %	0.257 ± 0.008^{b}	0.272 ± 0.011^{b}	0.298 ± 0.014^{a}
Total n6, %	2.750 ± 0.083^{b}	2.778 ± 0.056^{b}	2.894 ± 0.025^{a}
PUFA, %	3.710 ± 0.130^{b}	3.799 ± 0.077^{b}	3.990 ± 0.099^{a}

CON – control diet (concentrate + total mixed ration + hay), T1 – CON diet + 800 g of flaxseed, T2 – CON diet + 600 g of flaxseed + 200 g of chia seed; SFA – saturated fatty acids, MUFA – mono-unsaturated fatty acids, CLA – conjugated linoleic acids, PUFA – poly-unsaturated fatty acids; data are presented as mean value ± SEM; ^{abc} – means within a column with different superscripts are significantly different at $P < 0.05$

seeds on saturated fatty acid and polyunsaturated fatty acid contents, CLA increased in the T1 and T2 groups when compared to the control group ($P < 0.05$). Meanwhile, the concentration of n-3, n-6, and PUFAs was the highest in the T2 group ($P < 0.05$).

Figure 1 shows the effect of full-fat oil seed supplementation on volatile compound contents, antioxidant activity, and total cell count in raw milk. The supplementation of full-fat oil seeds did not affect the content of volatile compounds in raw milk; however, antioxidant activity significantly increased in the T2 group compared to the control group ($P < 0.05$). Moreover, full-fat oil seed addition did not alter the total cell count in raw milk.

Table 6 shows the effect of full-fat oil seeds supplementation on lipid oxidation in raw milk. As storage period at 4 °C and 15 °C was extended, lipid oxidation increased in all treatment groups, but the difference between them was small ($P < 0.05$) and lipid oxidation tended to occur faster at 15 °C than at 4 °C.

Table 6. Effect of full fat oil seeds on thiobarbituric acid reaction in milk during storage time (mg malondialdehyde/kg milk)

Storage temperature	Storage day	CON	T1	T2
4 °C	0	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.02
	3	0.38 ± 0.02	0.38 ± 0.03	0.50 ± 0.03
	6	0.49 ± 0.15	0.56 ± 0.09	0.71 ± 0.09
	9	0.59 ± 0.09	0.57 ± 0.11	0.86 ± 0.14
15 °C	0	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.02
	3	0.58 ± 0.08	0.49 ± 0.14	0.55 ± 0.15
	6	1.36 ± 0.30	1.23 ± 0.05	1.35 ± 0.03
	9	1.26 ± 0.34	1.01 ± 0.12	0.95 ± 0.26

CON – control diet (concentrate + total mixed ration + hay), T1 – CON diet + 800 g of flaxseed, T2 – CON diet + 600 g of flaxseed + 200 g of chia seed; data are presented as mean value ± SEM

Figure 2 shows the effect of the addition of full-fat oil seeds on oxygen concentration in raw milk. For non-pasteurized raw milk, oxygen levels in the T2 group showed an increasing tendency, compared to the control group, on day 3 and 5 of storage, at a storage temperature of 10 °C and illumination in-

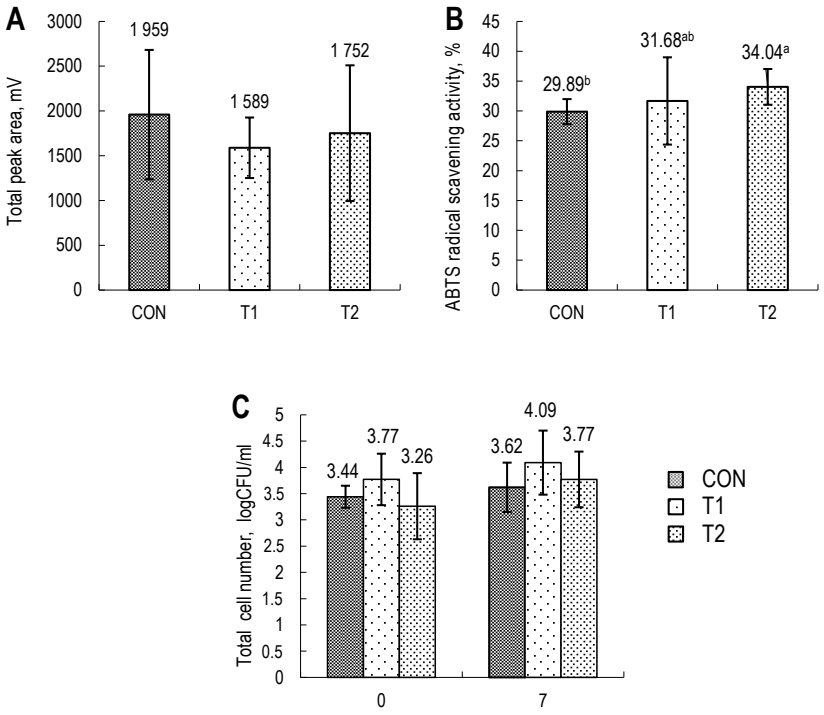

Figure 1. Effects of full fat oil seeds on volatile compounds content (A), antioxidant activity (B) and total cell number (C; 4 °C) in milk of Holstein cows

CON – control diet (concentrate + total mixed ration + hay), T1 – CON diet + 800 g of flaxseed, T2 – CON diet + 600 g of flaxseed + 200 g of chia seeds; ABTS – 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate); [ab] – means within a column with different superscripts are significantly different at $P < 0.05$

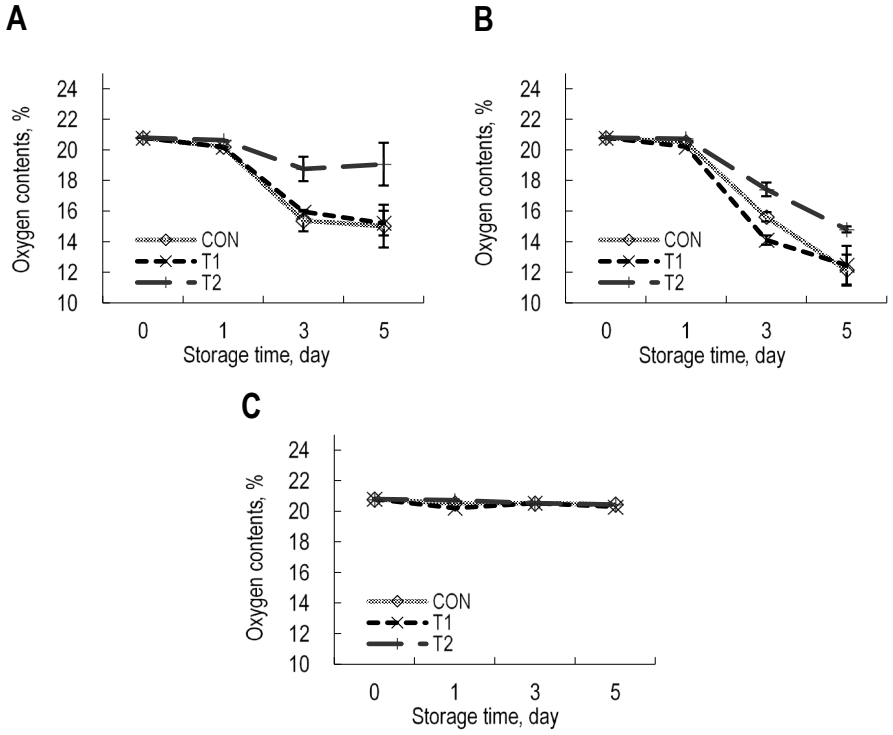

Figure 2. Effect of full fat oil seeds on oxygen content in milk during storage. (A) 2 200 lux/10 °C, (B) Darkroom/10 °C, (C) 2 200 lux/10 °C (heat treatment at 70 °C for 10 min)

CON – control diet (concentrate + total mixed ration + hay), T1 – CON diet + 800 g of flaxseed, T2 – CON diet + 600 g of flaxseed + 200 g of chia seeds; $P > 0.05$

tensity of 2 200 lux; however, this difference was not statistically significant. In darkroom culture, oxygen concentration in raw milk decreased steadily from day 3 to 5, regardless of the treatment group, whereas the level of oxygen in pasteurized raw milk was relatively constant from the initial day (day 0) to the 5th day without any differences between the treatment groups.

Discussion

In general, milk yield has been reported to vary depending on individual environmental and genetic factors (Miglior et al., 2007). In this study, the difference in milk yield between the treatment groups was small, but the milk yield during the second period, compared to the first period, was higher in the T1 (76–96%) and T2 (90–105%) groups than in the control group (80–95%). Therefore, supplementation of flaxseed or flaxseed + chia seeds could be considered beneficial for lactation persistency in Holstein cows.

The National Research Council (NRC, 2001) recommends using rumen bypass proteins and fats (energy) with high absorption rates to improve milk yield in lactating cows. Further, it has been reported that full-fat oil seeds are not sufficiently digested in the rumen due to insufficient mastication, but are instead transferred to the small intestine for digestion and absorption. In this study, the level of seed supplementation was not sufficient to increase milk yield, but we believe that rumen bypass proteins and energy supplies can prolong lactation and positively affect feed efficiency. Notably, Petit (2002) reported that supplementation with flaxseed increased milk yield compared to Megalac® for 16 weeks in lactating cows. Conversely, Ahn et al. (1998) found that chia seed supplementation tended to reduce milk yield. Therefore, it is considered that the effect of full-fat oil seeds on milk yield may vary depending on the level and duration of supplementation.

In general, lactating cows show differences in milk composition over time (Goff and Horst, 1997). It is believed that increases in milk protein and fat content are associated with lower milk yield towards the end of the lactation period.

In the present study, the effect of the addition of flaxseed or flaxseed + chia seeds on the overall milk composition was small. A similar trend was described by Ahn et al. (1998), who found no differences in milk protein content between lactating cows supplemented with corn silage or fish meal-flaxseed (3:7) for over 3 weeks. In addition, despite differences in the raw materials supplied, Mierlita et al. (2023) reported that supplementing the feed with oil or fat had little effect on increasing the milk fat content. Similarly, Neves et al. (2009) found that there was no change in milk lactose content after supplementing feed of lactating cows with extruded canola seeds. The results of these previous studies followed a similar trend to those obtained in the current work.

In lactating cows, the proportion of saturated fatty acids in milk has been reported to be high, as large amounts of unsaturated fatty acids in feed are converted into saturated fatty acids in the rumen by microbial biohydrogenation (Wang et al., 2002). In the present study, the proportion of saturated fatty acids was 72–73%, regardless of the treatment group, indicating that the supplementation of full fat oil seeds had little effect on the proportion of saturated fatty acids in lactating Holstein cows.

Moreover, CLA has been reported to be produced by incomplete biohydrogenation of linoleic acid in the rumen, which subsequently enters the small intestine and is transferred to tissues and milk (Kelly et al., 1998). The current and previous studies demonstrated that large quantities of linoleic acid contained in flaxseed or chia seed influenced the CLA content in milk. Dhiman et al. (2000) reported that supplementing feed or fat with a high content of linoleic acid effectively increased CLA concentration of milk, while Gonthier et al. (2005) reported that the proportion of CLA in milk fat increased when diets rich in linoleic acid (C18:2) and linolenic acid (C18:3) were fed. The results of the latter studies were consistent with findings of this work. In addition, the supplementation of flaxseed was shown to increase PUFA (Lessard et al., 2003) and n-3 fatty acid contents in milk (Schettino et al., 2017), while supplementation of chia seeds elevated PUFA (Goff and Horst, 1997) and CLA levels (Jun et al., 2005). According to the results of this study and other cited works, increased supplementation of flaxseed or chia seeds in lactating cows could effectively alter the fatty acid composition of milk, and the addition of flaxseed + chia seeds more efficiently increased the functional fatty acid content.

In general, volatile compounds detected in raw milk included acetaldehyde, ethanol, 2-propanone (acetone), and hexane (Desage et al., 1996). These compounds can be transferred to milk through the atmosphere, rumen gas, digestive system, and blood vessels, which has been reported to vary depending on the type of feed (Soong and Barlow, 2004). However, the results of this study showed no difference

in volatile compound contents between treatment groups, thus the effect of dietary full-fat oil seeds on the volatile compound contents in raw milk seemed to be small. Nevertheless, natural antioxidants have been isolated from plant materials such as full-fat oil seeds, grains, vegetables, and fruits (Choi et al., 1992); moreover, full-fat oil seeds containing large amounts of oil and fat have been reported to have a high content of antioxidants (Sargi et al., 2013). In particular, chia seeds contain large amounts of phenols and antioxidants (Ullah et al., 2016), and have been reported to exert beneficial health effects through antioxidant and anti-inflammatory activities (Kwon et al., 2019). Moreover, Dawson and Gartner (1983) found that chia seed extract significantly improved ABTS radical scavenging activity in yogurt, supporting the results of this study.

Unsaturated fatty acids in foods can readily bind oxygen due to their double bond structure (Chan, 1987), and the auto-oxidation of foods has been found to increases with the content of free fatty acids and degree of unsaturation (You and Lee, 1982). In addition, oxidative changes are carried out by lipolytic enzymes, hydrolysis, and microorganisms, and the longer the storage period and the higher the temperature, the faster the rate of lipid oxidation (Rashid et al., 2022). In the present study, lipid oxidation rapidly progressed under storage at 15 °C, which was consistent with previous research results. Further, this led to the conclusion that the supplementation of full-fat oil seeds had no effect on lipid oxidation.

Based on the results of this study, we do not believe that supplementation with full-fat oil seeds have an effect on oxygen concentration in raw milk. Furthermore, the change in oxygen concentration during storage under non-pasteurized conditions was greater than that observed during storage of the pasteurized product. This result could be attributed more to microbial influence than to differences in light intensity, temperature or fat composition.

Conclusions

The supplementation of flaxseed or flaxseed + chia seed was found in this study to have a positive effect on the fatty acid composition of milk without negatively affecting feed intake, milk production, and its quality in lactating Holstein cows. In particular, mixed supplementation of flaxseed and chia seeds is believed to have a positive effect on lactation persistency, functional fatty acid content (conjugated linoleic acid, polyunsaturated fatty acid, and n-3 fatty acid ratio), and antioxidant activity of milking cows, thereby supporting the production of functional milk.

Conflict of interest

The Authors declare that there is no conflict of interest.

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