Full Length Article

EFFECT OF DIETARY SUPPLEMENTATION OF RUMEN PROTECTED RAPESEED OIL ON MILK OMEGA-3 FATTY ACID PROFILE OF LACTATING CROSSBRED COWS

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ABSTRACT

The effect of rumen protected omega–3 fatty acids prepared from polyunsaturated fatty acids (PUFA) rich rapeseed oil on milk fatty acid profile of lactating crossbred cows have been studied in this experiment. Eighteen lactating crossbred cows were selected and divided randomly into three groups (GI, GII and GIII) with six animals in each group. All the animals in GI were fed with basal diet (concentrates and green roughage ad libitum). In GII and III group cows, rumen protected fatty acids were mixed with basal diet before feeding. The calcium fatty acids of rapeseed oil (CaRSO) and encapsulated rape seed oil were fed to GII and GIII group cows respectively, for 90 days of experimental period. The fatty acid profile of milk, rapeseed oil and bypass fat was analysed by gas chromatography. The supplementation of CaRSO and encapsulated rapeseed oil significantly (P<0.05) increased milk yield compared to control. Better production efficiency (P<0.01) and increased 4 per cent FCM (kg/day) was recorded in encapsulated fatty acid supplemented group compared to CaRSO. The total concentration of omega-3 fatty acids (alpha linoleic acid, eicosapentaenoic acid and docosahexaenoic acid) were detected in milk of treatment group cows compared to control group.

Keywords: Calcium soaps, Encapsulated, Lactating cows, PUFA's, Rapeseed oil, Rumen protected, Omega-3 fatty acids

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INTRODUCTION

In recent years, excessive consumption of medium-chain Saturated fatty acids (SFA) and trans fatty acids by human beings as risk factors for Cardiovascular disease (CVD) and in the etiology of insulin resistance (WHO, 2003; Shingfield *et al*., 2008; Kairenius *et al.,* 2015).–Intake of long-chain n-3 PUFA such as ALA (C18:3 n-3), Eicosapentaenoic acid (EPA; C20:5 n-3) and Docosahexaenoic acid (DHA; C22:6n-3), reduces risk of CVD,

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decreases the incidence of sudden cardiac arrest, improves immune function, and prevent cancers (Palmquist, 2009).

The essentiality of omega-3 fatty acids were proved that they down regulate the genes responsible for inflammation and obesity and increases the sensitivity to insulin as well as decreases VLDL and increases HDL in blood. This decreases the risk of chronic heart diseases in humans (Simopoulos, 2010). Hence, there has been considerable interest, to "designing" milk by less milk fat, altered milk fatty acid with CLA and ω-fats, increased essential amino acids and without β-lactoglobulin (β-LG) for the health benefit of human beings.

The designing of milk could be possible by dietary manipulations, high forage to concentrate ratio, or by feeding rumen protected calcium salts (Naik, 2013) encapsulated PUFA rich oils such as linseed oil, rapeseed oil, sunflower oil (Greco *et al*., 2015) and fish oil (Elis *et al*., 2015) or by feeding by-pass fat (Hundal *et al*., 2020), without affecting digestibility of dry matter and organic matter (Naik., 2013; Subrahmanyeswar *et al*., 2019). However, apparent transfer efficiency of eicosapentaenoic acid and docosahexaenoic acid from feed to milk is very low and ranged from 1.9 to 3.3 per cent (Or-Rashid *et al.,*2009), which related to their extensive bio-hydrogenation in the rumen. Hence, the present study aims to ascertain the effect of supplementation of rumen protected omega-3 fatty acids prepared from rapeseed oil on milk yield, and milk omega-3 fatty acid profile of lactating crossbred cows.

MATERIALS AND METHODS

Preparation of rumen protected fatty acids

Rumen protected omega-3 fatty acids were prepared from rapeseed oil in Animal Nutrition laboratory by double decomposition process following the Naik *et al.* (2007) and Perez *et al*. (2009) with modifications. In this process, equal quantity of hot rapeseed oil was mixed with calcium oxide (CaO) (equal number of moles of calcium oxide for saponification) at 50°C (heating temperature should be slightly above melting point) then glycerol (6%) as emulsifying agent was added. Equal quantity of water was also added and heated in pressure cooker at 2 kg psi for one hour to complete saponification process. The saponified rapeseed oil with calcium salts was allowed to cool and powdered for use.

The encapsulation of rapeseed fatty acids was prepared following Gawad *et al*. (2015) method. The rapeseed oil and sodium alginate gel were mixed at 0.8:1 ratio, emulsifying agent Tween-80 was added with this gel mixture and 2.5 per cent calcium chloride was mixed thoroughly for cross linking effect. Sodium alginate gel was prepared by dissolving sodium alginate powder in water (1:4 w/w). The encapsulated RSO was semisolid and was stored in deep-freezer till fed to the animals.

Animals and feeding experiment

The feeding experiment was carried out in Cattle Farm, Instructional Livestock Farm complex (ILFC), College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala, from August to November

2018. Eighteen cross bred lactating dairy cows, within two months of lactation were selected and they were divided into three groups of six animals each following completely randomized design.

All the animals were maintained in individual stalls with sufficient space, ventilation and automatic watering facilities. The cows in control group (GI) were fed with basal feed with 22 per cent CP and 70 per cent TDN. All the cows were offered *ad libitum* green grass. The cows in treatment group G II were fed with basal feed supplemented with protected fat prepared by double decomposition method at 20 g/kg milk/day and cows in treatment group G III were fed with basal feed supplemented with protected fat prepared by encapsulation method at 20 g/kg milk/day. The cows were offered concentrate mixture daily at 5:30 AM and 1:30 PM and green grass daily at 8:30 AM, 2:00 PM and at evening 6:00 PM to meet their nutrient requirements as per ICAR (2013) recommendation for optimum milk production and dry matter (DM) requirement. The quantity of concentrate mixture and bypass fat required by each lactating cow were adjusted at every fifteen days interval based on their milk production. The experimental feeding lasted for a period of 90 days. The data on daily dry matter intake (DMI), milk yield were recorded.

Feed and milk analysis

Samples of feed, bypass fat were analysed for proximate principles, calcium and phosphorus as per the methods of AOAC (2016). The individual animal's milk samples were collected at weekly intervals and pooled samples were analysed at monthly interval for milk fat (IS: 1223:2001).

Fatty acid profile of milk, rapeseed oil and rumen protected fat

Fifty mL of milk was collected in the morning and evening from individual animals directly into centrifugation tubes on 30th, 60th and 90th day of experiment and stored in deep freezer at -20 °C. The fatty acid (FA) profile of milk fat was separated by using n-hexane and diethyl ether (Luna *et al*., 2005) and extracted by method described by Hara and Radin (1978). Fatty acid methyl ester (FAME) was prepared as per Morrison and Smith (1964). However, FA profile in rapeseed oil, protected fat samples were estimated following Czauderna and Kowalczyk, (2001), Cieślak *et al*. (2009) and AOAC (2016) methods.

The fatty acids prepared as methyl ester was analysed by using gas chromatograph (GCMS-QP2010 Ultra, Shimadzu, Japan) equipped with an auto sampler, a flame ionization detector. A capillary column (100 m length x 0.25 mm internal diameter, 0.20 µm; Rt-2560 Restek®) was used for analysis. The high purity carrier gas helium (99.9999 per cent) with a total flow rate of 106.7 ml/min and a column flow rate of 1 mL/min was maintained. The sample volume was 1 µL with a split ratio of 1:100 and oven temperature program was initially set at 100 ºC which was held for 4 min, then ramped at 3 ºC/min to 190 ºC and held for 5.0 min, then ramped at 2 ºC/min to 230 ºC and held for 20 min. The total run time was 79 min. The injection port and flame ionization detector temperatures were programmed at 225ºC, and 245ºC, respectively. Standard mixture (C4-C24; Food Industry FAME Mix, Restek® USA, Cat no. 35077) and Linolenic acid methyl ester isomer mix, Methyl all-cis-5,8,11,14,17-Eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-Docosahexaenoic acid (DHA) (Sigma-Aldrich, India) were used as reference for quantification. Fatty acids results were quantified as g fatty acid per 100 g of fats.

Statistical analysis

Data collected on various parameters were subjected to statistical analysis using SPSS version 21.0 using one way ANOVA method as per described by Snedecor and Cochran, (1994).

RESULTS AND DISCUSSION

Nutrient composition of experimental ration and dry matter intake

The nutrient composition of experimental ration used in this study was presented in Table 1. The estimated values for DHA and ALA were below the detected level in experimental rations. The other nutrients provided to the animals were well balanced. However, the daily intake of nutrients were adjusted at every 15 days interval based on their milk yield (MY) and only concentrate was fed extra. DMI (kg/day) recorded in this study was 10.10 ±0.06 in GI, 10.57 ±0.062 in GII and significantly (P<0.01) higher in GIII (11.50 ± 0.06) animals. This increased DMI indicated, that the dietary inclusion of rumen protected rapeseed oil fatty acids (200 g/day/animal) did not affect the palatability of the concentrate fed. However, high-erucic acid, low glucosinolate in rapeseed meal decreased the feed intake of dairy animals which affected their milk yield (MY) (Hristov *et al*., 2011**).**

Milk yield, milk fat and production efficiency

The average milk yield (MY) (kg/day) reported in this experiment indicated that rumen protected fat supplemental group animals showed significantly (P<0.05) increase in MY (10.65±0.09 and 10.65±0.14 respectively in GII and GIII) compared to control (10.34±0.04). Milk fat (%) reported in this study was similar in GI (4.23 ± 0.121) and GIII (4.56 ± 0.121), but value recorded in GII (3.80 ± 0.126) group animals was significantly (P<0.01) lesser than the other groups.

The production efficiency in terms of MY (kg/day) per kg of DMI was better (P<0.01) in GII (1.02±0.009) animals than GIII (0.91±0.009). Meanwhile, other important parameter 4 per cent FCM (kg/day) was significantly (P<0.01) better in encapsulated FA supplemented group (GIII) (11.18±0.15) compared to CaRSO (10.75±0.10) and control (10.55±0.05) group animals. These results are in agreement with Gowda *et al.* (2013); Kowalski *et al*. (1999) and Naik *et al*. (2009) reported as significant (P<0.05) increase in MY in dairy cows. In contrary, to our findings Purushothaman *et al*. (2008) reported protected fat supplementation caused poorer feed efficiency, whereas, Ranjan *et al*. (2013) reported increased (P<0.05) feed efficiency. The increase in MY and production efficiency in protected fat supplemental group animals might be due to additional energy supplied by rumen protected rapeseed oil as per Subrahmanyeswar *et al*. (2019).

Fatty acid profile of rapeseed oil and protected fat

The fatty acid profile of rapeseed oil and protected fat estimated in this experiment was presented in Table 2. The estimated FA in this research indicated rapeseed oil contained 10.61 per cent SFA, 63.02 per cent MUFA and 26.37 per cent of PUFA.

These results are in confirmation with other findings for PUFA ranged from 20.9 to 29.0 per cent (Lindman, 2015; Orsavova *et al*., 2015). Estimated PUFA values of rapeseed oil in this study (g/100 g fat) were 6.839 of ALA (C18:3 n-3); 0.756 of EPA (C20:5 n-3) and 0.078 of DHA (C22:6 n-3) similar to the reported values of Sudharsan *et al.* (2021) and corroborates with other researchers findings for ALA ranged from 8.0 to 11.1 g/100 g fat (Glasser *et al*., 2008; McDonald *et al*., 2010; Ghazani *et al*., 2013).

The concentration of erucic acid estimated in this study was 43.736 g/100 g fat in rapeseed oil, agreement with the present findings as reported by Mortuza *et al*. (2006) erucic acid (C22:1, n-9) content of rapeseed cultivars ranged from 21.59 to 51.57 per cent in Bangladesh. The variation in the fatty acid profile might be due to different cultivars of Brassica, cultivation location, effect of season and processing techniques implied for extraction of oil. The oil used in this study was collected from *Brassica napus* variety cultivated in West Bengal, India.

Fatty acid profile of milk fat

The fatty acid profile of milk fat of experimental cows supplemented with protected fat as CaRSO and encapsulated FA was presented in Table 3.

The influence of continuous feeding of protected fat for 90 days, was recorded in this study, revealed changes in omega-3 fatty acids composition of milk fat. The concentration of EPA was only detected in milk fat of control group and ranged the levels from 0.672 to 1.002 (g/100 g fat). While comparing between CaRSO and encapsulated FA supplementation in the present study, higher value of ALA were recorded in CaRSO group. These findings were supported by Enjalbert *et al*. (1997); Kowalski *et al*. (1999) and Hoffmann *et al*. (2016) who supplemented dairy cows with calcium soaps of rapeseed oil fatty acids.

Similarly, other two omega-3 fatty acids, EPA & DHA were detected in higher concentration in encapsulated FA (GIII) supplemented group animal's milk fat compared to CaRSO fed animals. These findings were in accordance with Elis *et al*. (2015) who mentioned protection of fish oil by encapsulation process increased concentration of EPA and DHA in dairy cows. Meanwhile, Hundal *et al*. (2020) reported 0.013 per cent of EPA and 0.015 per cent of DHA in dairy cows supplementation with bypass fat. There was no literature with knowledge of authors to compare the effect of double decomposition (CaRSO) and encapsulation method.

Results indicated that supplementation of PUFA rich rapeseed oil in rumen protected form had increased the dietary intake of PUFA's (Elis *et al*., 2015; Hoffmann *et al*., 2016), which influenced *de novo* fatty acid synthesis in mammary gland and increased the proportions of omega-3-fatty acids and

Table 1. Nutrient composition of experimental rations

Nutrients	Concentrate	Green grass	Experimental rations		
			GI	GII	GIII
Proximate composition % DM					
Dry Matter	86.87±0.43	26.55±0.38	47.93±0.6	51.84±0.17	52.45±0.13
Crude Protein	22.03±0.13	10.52±0.15	14.36±0.05	15.48±0.22	15.69±0.15
Crude Fibre	13.27±0.51	36.15±0.26	31.11±0.23	27.79±0.29	29.25±0.33
Ether extract	2.34±0.10	1.43±0.036	2.07±0.05	3.57±0.021	3.14±0.061
Total Ash	12.31±0.14	8.19±0.18	5.04±0.01	6.13±0.039	6.07±0.03
Nitrogen free extract	50.06±0.69	43.71±0.34	47.42±0.13	47.03±0.64	45.86±0.28
Fatty Acid Profile (g/100 g fat)					
Methyl dodecanoate (C12:0)	0.560	0.360	BDL	0.380	BDL
Methyl pentadecanoate (C 15:0)	0.828	BDL	0.534	0.576	0.469
Methyl alpha-Linolenic acid (Cis-9,12,15) C18:3	BDL	0.385	--	--	---
Eicosapentaenoic acid (Cis-5,8,11,14,17) C20:5	0.197	0.230	BDL	0.228	0.107
Arachidonic acid (Cis-5, 8, 11, 14) C20:4	2.048	BDL	0.361	BDL	BDL
Cis-4, 7, 10, 13,16, 19-Docosahexaenoic acid (C22:6)	0.013	--	---	--	--

BDL- Below detectable limit

Table 2. Fatty acid profile of rapeseed oil & rumen protected fat, g/100 g fat

Poly Unsaturated Fatty Acids	Rapeseed Oil	Protected Fat supplement	
		CaRSO	Encapsulated fat
Linoleicacid/ 9,12-octadecadienoic acid(Cis-9,12) C18:2 (n-6)	BDL	25.694	8.857
Methyl gama- Linolenic acid (Cis-6,9,12) C 18:3 (n-6)	1.987	5.088	BDL
cis-9,cis-12, cis-15-octadecatrienoic acid C 18:3 (n-3)	6.839	5.043	BDL
Methyl eicosadienoate (Cis-11, 14) C 20:2	2.388	--	--
Methyl eicosatrienoate (Cis-8, 11,14) C 20:3	2.841	6.506	BDL
Arachidonic acid (Cis-5, 8, 11, 14) C20:4	2.738	2.019	1.318
Eicosapentaenoic acid (Cis-5,8,11,14,17) C20:5	0.756	BDL	0.129
Cis-4, 7, 10, 13,16, 19- Docosahexaenoic acid (C22:6)	0.078	--	--

BDL- Below detectable limit

Table 3. Fatty acid profile **of milk fat of experimental cows supplemented with rumen protected fat**

Fatty Acids	G I (control)			GII (Ca RSO Group)			GIII (Encapsulated Group)			P -Value
	I month	II month	III month	I month	II month	III month	I month	II month	III month	
C18:3 (n-3)	BDL	BDL	BDL	BDL	2.412± 1.37	2.136± 1.20	1.208± 0.32	2.094± 0.51	BDL	--
C 18:3 (n-3) (ALA)	BDL	BDL	BDL	BDL	BDL	0.501± 0.00	BDL	0.422± 0.00	0.495± 0.00	---
C20:5 EPA (n-3)	1.002± 0.07	0.672± 0.08	0.931± 0.19	1.038± 0.220	0.850± 0.10	BDL	1.829± 1.039	BDL	0.977± 0.174	---
C22:6 DHA (n-3)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.515± 0.000	---
C18:2 (trans LA 9,12) n-6 ^{ns}	2.783± 0.54	2.215± 1.15	4.656± 1.28	5.989± 2.35	4.293± 1.23	4.624± 1.32	3.408± 0.70	6.540± 1.04	4.898± 1.12	0.39
C18:2 (Cis 9,12) n-6 ^{ns}	20.903± 7.09	BDL	12.815± 7.02	17.601± 5.33	10.435± 2.38	9.041± 0.00	12.357± 8.38	14.497± 0.0	47.925± 2.99	0.459
C 18:3 n-6 ^{ns}	4.156± 0.601	5.337± 0.81	6.479± 1.16	5.935± 0.965	4.342± 1.15	4.540± 1.24	4.300± 0.42	6.340± 0.71	4.709± 0.65	0.982
Σ Omega 3 FA	1.002± 0.07	0.672± 0.08	0.931± 0.19	1.038± 0.220	3.262± 0.520	2.637± 1.20	3.037± 0.68	2.516± 0.51	1.987± 0.17	---
Σ Omega 6 FA	27.842± 8.15	8.075± 0.68	23.95± 2.77	29.525± 2.88	19.07± 1.59	18.21± 2.56	20.065± 3.17	27.37± 0.58	57.532± 3.13	---

BDL- Below detectable limit

PUFA's in the milk (Williams, 2000 and Chilliard and Ferlay, 2004). Further, Gowda *et al*. (2013) mentioned there was an increase in milk yield in experimental cows.

. Several recent research works had documented the biochemistry and mammary lipogenesis in ruminants explained that mammary epithelial cells synthesized short (C4:0 to 12:0) and medium-chain (C14:0 to C16:0) fatty acids *de novo* and influenced the concentration of other long chain FA in milk fat. However, other C18 FA in milk fat are absorbed from the small intestine (Harvatine *et al*., 2009; Shingfield *et al*., 2013).

The research findings of this study, increased concentration of C18:2, n-6 and C18:3 n-3 FAs (Table 3) were corroborated with report of post ruminal infusion of linseed oil which increased bovine milk fat at 16.6 and 25.4 g/100 g fatty acids and also indicated that 18:2 n-6, and 18:3 n-3 were transferred from the small intestine into milk fat with a mean efficiency of 49 per cent (Shingfield *et al*., 2013).

CONCLUSION

The dietary supplementation of CaRSO and encapsulated rapeseed oil in protected form increased milk yield and production efficiency. Comparatively, encapsulation of rapeseed oil FA's showed promising results with detectable concentration of omega-3 fatty acids compared to Calcium salts of rapeseed oil.

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