Effects of different vegetable oils on rumen fermentation and conjugated linoleic acid concentration in vitro

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Abstract

Aim: The objective of this study was to investigate the effect of different vegetable oils on rumen fermentation and concentrations of beneficial cis-9 trans-11 CLA and trans-11 C18:1 fatty acid (FA) in the rumen fluid in an in vitro condition.

Materials and Methods: Six vegetable oils including sunflower, soybean, sesame, rice bran, groundnut, and mustard oils were used at three dose levels (0%, 3% and 4% of substrate dry matter [DM] basis) in three replicates for each treatment in a completely randomized design using 6 x 3 factorial arrangement. Rumen fluid for microbial culture was collected from four goats fed on a diet of concentrate mixture and berseem hay at a ratio of 60:40 on DM basis. The in vitro fermentation was performed in 100 ml conical flasks containing 50 ml of culture media and 0.5 g of substrates containing 0%, 3% and 4% vegetable oils.

Results: Oils supplementation did not affect (p>0.05) in vitro DM digestibility, and concentrations of total volatile FAs and ammonia-N. Sunflower oil and soybean oil decreased (p<0.05) protozoal numbers with increasing levels of oils. Other oils had less pronounced effect (p>0.05) on protozoal numbers. Both trans-11 C18:1 FA and cis-9, trans-11 CLA concentrations were increased (p<0.05) by sunflower and soybean oil supplementation at 4% level with the highest concentration observed for sunflower oil. The addition of other oils did not significantly (p>0.05) increase the trans-11 C18:1 FA and cis-9, trans-11 CLA concentrations as compared to the control. The concentrations of stearic, oleic, linoleic, and linolenic acids were not altered (p>0.05) due to the addition of any vegetable oils.

Conclusion: Supplementation of sunflower and soybean oils enhanced beneficial trans-11 C18:1 FA and cis-9, trans-11 CLA concentrations in rumen fluid, while sesame, rice bran, groundnut, and mustard oils were ineffective in this study.

Keywords: conjugated linoleic acid, goat, rumen fluid, vaccenic acid, vegetable oil.

Introduction

Enrichment of the nutraceutical quality of meat and milk of ruminant origins has been of growing interests among the researchers using dietary approaches due to increasing demands of healthy foods by the consumers [1-4]. The healthy fatty acids (FA), especially conjugated linoleic acids (CLAs) and n-3 polyunsaturated FA (eicosapentaenoic acid and docosahexaenoic acid) in foods for human consumption have shown several potential health benefits in several studies [1,5]. Another FA, trans-11 C18:1 (also called vaccenic acid; VA) is also associated with increased risks of cardiovascular disease [6,7]. Milk and meat from ruminants are the main natural sources of CLAs [8]. However, usual dietary intakes of meat and milk are not adequate in fulfilling the requirement of cis-9, trans-11 CLA to achieve expected health benefits [1]. Therefore, several studies over the last two decades have been conducted for enhancing the cis-9, trans-11 CLA content in milk and meat of ruminants by supplementing linoleic and linolenic acid rich oils and oil seeds, which increase the availability of precursors of CLA synthesis [9,10], and modulating rumen microbiota and metabolism responsible for biohydrogenation of unsaturated C18 FA to stearic acid [2,3].

The cis-9, trans-11 CLA in meat and milk is partly absorbed from the gut after partial biohydrogenation of linoleic acid in the rumen [9]. The major part of this CLA is synthesised endogenously by the enzyme delta-9-desaturase in the animal tissues from VA, which is also a biohydrogenation intermediate of oleic, linoleic, and linolenic acids [11,12]. Different oils differ in their ability to increase the concentrations of these beneficial FA in rumen fluid and subsequently in meat and milk.

Therefore, this study was conducted to investigate the effects of different types of vegetable oils on rumen fermentation and concentration of CLAs and VA in rumen fluid in vitro.

Materials and Methods

Ethical approval

The experiment was approved by the Institutional Animal Ethics Committee for Animal Care and
Management, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India.

Experimental design

Six different vegetable oils including sunflower, soybean, sesame, rice bran, groundnut, and mustard oils were procured from local grocery stores. These vegetable oils were used at three dose levels (0%, 3%, and 4% of substrate on dry matter [DM] basis) in three replicates for each treatment in a completely randomized design with a 6 × 3 factorial arrangement. Low concentrations of these oils as precursors of CLA in biohydrogenation process may not increase the CLA concentration in rumen fluid, while high concentration could inhibit overall rumen fermentation process [13]. We hypothesized that oils at 3-4% levels may enhance CLA concentration without affecting rumen fermentation.

Rumen incubation

Rumen liquor was collected by a stomach tube from four goats fed on a diet of concentrate mixture and berseem hay at a 60:40 ratio on DM basis. The rumen liquor was collected during morning before feeding and watering, transported in insulated flasks under anaerobic conditions to the laboratory, pooled in equal proportions and used as a source of inoculums. The fermentation process was conducted in 100 ml conical flasks containing 50 ml of culture media (1:4 ratio of rumen fluid and phosphate-bicarbonate buffer [14], purged with CO₂), 0.5 g of substrates supplemented with 0%, 3%, 4% of each vegetable oil. Concentrate mixture (crude protein - 17.4%; neutral detergent fiber - 38.1%; ether extract - 1.12% on DM basis) and berseem hay (crude protein - 13.1%; neutral detergent fiber - 67.2%; ether extract - 1.21% on DM basis) at 60:40 ratio on DM basis were used as substrates. After flushing CO₂ in the flasks for 5 min, a cork fitted with Bunsen gas release valve was tightly placed over the chamber following the procedure of Kamra et al. [16].

For protozoa count, 1 ml sample was mixed with 0.5 ml methyl green formal saline solution. The stained sample was kept overnight, and protozoal numbers were counted microscopically using Neubauer counting chamber following the procedure of Barnett and Reid [17]. Ammonia-N was estimated by the micro-Kjeldahl method [18].

FA concentrations in feeds, vegetable oils, and rumen fluid were measured for control and 4% oil supplemented samples following the method of O’Fallon et al. [19] with slight modification, which has also been described previously [3]. 20 μl sample was placed into a 16 mm × 125 mm screw-cap Pyrex culture tube to which 0.5 ml of the C13:0 internal standard (0.5 mg of C13:0/mL of methanol), 0.35 ml of 10 N KOH in water, and 2.65 ml of MeOH were added. The tube was incubated at 55°C for 1.5 h with vigorous handshaking for 5 s every 20 min to properly permeate, dissolve and hydrolyze the FA in the samples. After cooling below room temperature in a cold tap water bath, 0.29 ml of 24 N H₂SO₄ in water was added. The tube was mixed by inversion and with precipitated K₂SO₄ present was incubated again at 55°C for 1.5 h with hand-shaking for 5 s every 20 min. After FA methyl ester (FAME) synthesis, the tube was cooled in a cold tap water bath, 1.5 ml of hexane was added, and the tube was vortex-mixed for 5 min on a multi-tube vortex. The tube was centrifuged for 5 min in a tabletop centrifuge at 2500 rpm, and the hexane layer, containing the FAME, was placed into a vial. The vial was capped and placed at −20°C until analysis. Concentrations of FA in the samples were analyzed in a gas chromatography fitted with capillary column (100 m × 0.25 mm × 0.20 μm). Helium was used as a carrier gas. FAs were identified by comparing their retention time with the FAME standard.

Statistical analysis

The data analysis was performed by SPSS, version 16 [20] software. Rumen fermentation and FA concentration data were analyzed in two-way ANOVA with oil type, dose levels and their interaction as the main effects in 6 × 3 and 6 × 2 factorial arrangements, respectively. No variable except protozoal counts was affected (p>0.05) by the interaction effect. Then, data were analyzed in one-way ANOVA among the dose levels and oil type. Tukey’s test was used to find out the differences among the dose levels.

Results

Mustard oil contained the highest concentration of C18:3 FA, while C18:2 FA content was higher in sunflower oil, followed by soybean oil (Table-1). Rice bran oil was richest in C18:1 and C16:0 FA. Ruminal fermentation parameters are presented in Table-2. Oils supplementation at 3% and 4% level did not influence IVDM, pH, and concentrations of total VFA and ammonia-N in rumen fluid. Protozoal counts were affected by oil × level interaction. Sunflower oil and soybean oil decreased (p<0.05) protozoal numbers with increasing levels of oils (Table-3). Other oils had less pronounced effect on protozoal numbers. The effects of different vegetable oils on FA concentrations in rumen fluid are presented in Table-4. Both VA (trans-11 C18:1) and cis-9, trans-11 CLA concentrations were improved (p<0.05) by sunflower and soybean oil supplementation at 4% level. The increment of these FA was greater for sunflower oil. Addition of other oils did not significantly increase the VA concentrations as
Table 1: FA composition of vegetable oils and substrate (g/100 g of total FAs).

| FA          | Rice bran | Soybean | Sesame | Mustard | Sunflower | Groundnut | Concentrate | Berseeem |
|-------------|-----------|---------|--------|---------|-----------|-----------|-------------|----------|
| C14:0       | 0.39      | 0.09    | 0.03   | 0.14    | 0.33      | 0.08      |             |          |
| C16:0       | 20.7      | 12.2    | 9.73   | 4.16    | 8.50      | 13.1      |             |          |
| C16:1       | 0.23      | 0.08    | 0.24   | 0.23    | 0.33      | 0.14      |             |          |
| C18:0       | 2.66      | 3.27    | 5.10   | 1.83    | 7.40      | 4.00      |             |          |
| C18:1       | 40.0      | 29.4    | 37.2   | 13.6    | 25.7      | 43.2      |             |          |
| C18:2       | 34.4      | 45.8    | 39.6   | 34.0    | 54.2      | 35.8      |             |          |
| C18:3       | 0.56      | 3.78    | 1.57   | 9.28    | 0.32      | 0.74      |             |          |

Fatty acid

Table 2: Effects of vegetable oil supplementation on rumen fermentation in the rumen fluid after 24 h of incubation.

| Vegetable oil | Dose level | SEM | p value |
|---------------|------------|-----|---------|
|               | 0%         | 3%  | 4%      |          |
| IVDMD (%)     |            |     |         |
| Mustard       | 46.4       | 45.8| 45.8    | 0.65     | 0.729    |
| Groundnut     | 45.9       | 45.2| 45.0    | 0.26     | 0.351    |
| Sunflower     | 45.0       | 44.8| 43.6    | 0.36     | 0.207    |
| Sesame        | 46.0       | 45.8| 45.7    | 0.35     | 0.531    |
| Soybean       | 45.2       | 44.3| 43.2    | 0.58     | 0.185    |
| Rice bran     | 45.9       | 44.8| 46.5    | 0.22     | 0.296    |
| SEM           | 0.23       | 0.61| 0.89    |          |          |
| p value       | 0.897      | 0.424| 0.479  |
| Total VFA (mmol/dl) |        |     |         |
| Mustard       | 5.58       | 5.42| 5.50    | 0.05     | 0.640    |
| Groundnut     | 5.42       | 5.38| 5.44    | 0.04     | 0.554    |
| Sunflower     | 5.37       | 5.36| 5.15    | 0.05     | 0.361    |
| Sesame        | 5.33       | 5.55| 5.49    | 0.09     | 0.846    |
| Soybean       | 5.42       | 5.28| 5.46    | 0.08     | 0.262    |
| Rice bran     | 5.39       | 5.45| 5.32    | 0.05     | 0.450    |
| SEM           | 0.07       | 0.08| 0.10    |          |          |
| p value       | 0.778      | 0.692| 0.623  |
| pH            |            |     |         |
| Mustard       | 6.67       | 6.63| 6.53    | 0.03     | 0.813    |
| Groundnut     | 6.53       | 6.56| 6.67    | 0.04     | 0.732    |
| Sunflower     | 6.60       | 6.57| 6.40    | 0.05     | 0.708    |
| Sesame        | 6.53       | 6.53| 6.50    | 0.02     | 0.892    |
| Soybean       | 6.53       | 6.47| 6.50    | 0.04     | 0.655    |
| Rice bran     | 6.53       | 6.63| 6.56    | 0.04     | 0.647    |
| SEM           | 0.08       | 0.05| 0.06    |          |          |
| p value       | 0.904      | 0.178| 0.754  |
| Ammonia-N (mg/dl) |       |     |         |
| Mustard       | 8.32       | 8.09| 8.27    | 0.05     | 0.424    |
| Groundnut     | 8.07       | 8.20| 8.30    | 0.06     | 0.375    |
| Sunflower     | 8.07       | 8.20| 8.30    | 0.06     | 0.408    |
| Sesame        | 8.31       | 8.39| 8.16    | 0.06     | 0.666    |
| Soybean       | 8.38       | 8.06| 8.26    | 0.07     | 0.307    |
| Rice bran     | 8.34       | 7.96| 8.09    | 0.08     | 0.266    |
| SEM           | 0.09       | 0.08| 0.05    |          |          |
| p value       | 0.670      | 0.512| 0.540  |

Table 1: FA composition of vegetable oils and substrate (g/100 g of total FAs).

IVDMD=In vitro dry matter digestibility, VFA=Volatile fatty acids, SEM=Standard error of mean

compared to the control. The concentrations of stearic, oleic, linoleic, and linolenic acids were not (p>0.05) altered due to addition of any vegetable oils.

Discussion

Oil supplementation sometimes exerts detrimental effects on digestibility and VFA production due to general inhibitory effect of oils on rumen microbiota [21]. In this study, rumen fermentation was not affected, which was likely due to low concentration of oils used. Usually, oils or fats at concentrations of 4% in the diet do not affect rumen fermentation and may improve production performance of ruminants [13,22]. Soybean oil at 6% of diet did not influence DM degradability and total VFA concentrations in vitro [23]. Soybean oil-fish oil and rapeseed-fish oil blends reduced IVDMD when concentrations of oil were >5% of the diet, but not at lower concentration [24]. Sunflower oil and soybean oil inhibited the growth of protozoa, which was also observed in other studies [21] and is influenced by the degree of unsaturation of FA with greater unsaturation causing higher inhibitory effects on protozoa. Despite inhibition of protozoa, which may lower ammonia concentration by sunflower oil and soybean oil, ammonia concentration was not changed. This was probably due to lower time of incubation and low dose of oils to influence ammonia concentration by low number of protozoa. Gómez-Cortés et al. [23] reported that supplementation of soybean oil at 6% of the diet tended to decrease ammonia concentration in vitro.

Vegetable oils rich in C18:2 cis-9, cis-12 (linoleic acid) and C18:3 cis-9, cis-12, cis-15 (linolenic acid) FA could potentially increase VA and CLA concentration in the rumen fluid by bacteria biohydrogenation [10]. Linoleic acid has been shown to be converted to C18:2 cis-9, trans-11 and C18:1 cis-9, trans-11 while linolenic acid could be converted to cis-9, trans-11, cis-15 18:3 conjugated triene, then to trans-11, cis-15 18:2, and finally to an octadecenoic acid that is either trans-11, cis-15 18:2, or cis-15 18:1 via rumen biohydrogenation [25]. The supplementation of soybean oil and sunflower oil enhanced VA and cis-9, trans-11 CLA concentrations in rumen fluid to a great extent. El-Sherbiny et al. [24] also found that VA and cis-9, trans-11 18:2 concentrations in rumen fluid were increased by supplementation of soybean and rapeseed oil at 5% of DM, but not at 3% of DM. In another study, concentration of cis-9, trans-11 CLA was not altered, but the concentration of VA was increased in the in vitro rumen fluid by supplementation of soybean-fish oil blend at 3% of diet [26]. From this study and other studies, it appears that supplementation of vegetable oils at 4% or greater levels would be needed.

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Table 3: Effects of vegetable oil supplementation on protozoal population in the rumen liquor after 24 h of incubation.

| Vegetable oil | Dose level | SEM | p value |
|---------------|------------|-----|---------|
|               | 0% | 3% | 4%   | Oil | Dose level | Oil×dose |
| Mustard       | 36.2 | 35.2<sup>a</sup> | 33.5<sup>b</sup> | 1.21 | 0.264 | 0.005 | 0.108 |
| Groundnut     | 35.3 | 36.2<sup>a</sup> | 34.7<sup>b</sup> | 0.61 |       |       |       |
| Sunflower     | 36.1<sup>c</sup> | 31.5<sup>c</sup> | 27.2<sup>c</sup> | 1.51 |       |       |       |
| Sesame        | 36.3 | 34.9<sup>a</sup> | 34.3<sup>a</sup> | 1.03 |       |       |       |
| Soybean       | 36.2<sup>c</sup> | 33.2<sup>c</sup> | 30.9<sup,c</sup> | 1.19 |       |       |       |
| Rice bran     | 36.6 | 33.1<sup>c</sup> | 32.2<sup>c</sup> | 0.91 |       |       |       |
| SEM           | 0.25 | 1.15 | 1.40 |     |       |       |       |
| p value       | 0.844 | 0.042 | 0.027 |     |       |       |       |

<sup>a,b</sup>Means with different superscript letters within a row differ significantly (p<0.05). <sup>c</sup>Means with different superscripts letters within a column differ significantly (p<0.05). SEM=Standard error of mean, CLA=Conjugated linoleic acid, VA=Vaccenic acid, FA=Fatty acid

Table 4: Effect of vegetable oil supplementation on FA profile (% of total FAs) in the rumen fluid after 24 h of incubation.

| Vegetable oil | Dose level | SEM | p value |
|---------------|------------|-----|---------|
|               | 0% | 4%   |       |
| Stearic acid (C18:0) |       |       |       |
| Mustard       | 25.3 | 26.7 | 0.50 | 0.464 |
| Groundnut     | 25.4 | 24.7 | 0.41 | 0.322 |
| Sunflower     | 26.4 | 23.1 | 0.80 | 0.160 |
| Sesame        | 26.2 | 25.8 | 0.60 | 0.377 |
| Soybean       | 25.9 | 23.4 | 0.70 | 0.153 |
| Rice bran     | 25.9 | 28.2 | 1.22 | 0.157 |
| SEM           | 0.28 | 1.16 |     |       |
| p value       | 0.816 | 0.408 |     |       |
| Oleic acid (cis-9 C18:1) |       |       |       |
| Mustard       | 15.5 | 18.2 | 0.90 | 0.106 |
| Groundnut     | 15.8 | 18.3 | 0.64 | 0.089 |
| Sunflower     | 16.7 | 13.9 | 1.30 | 0.133 |
| Sesame        | 16.7 | 18.3 | 0.44 | 0.219 |
| Soybean       | 16.7 | 15.6 | 1.00 | 0.431 |
| Rice bran     | 17.0 | 14.4 | 0.80 | 0.136 |
| SEM           | 0.46 | 1.53 |     |       |
| p value       | 0.613 | 0.340 |     |       |
| VA (trans-11 C18:1) |       |       |       |
| Mustard       | 6.57 | 6.95<sup>c</sup> | 0.13 | 0.709 |
| Groundnut     | 6.66 | 7.10<sup>c</sup> | 0.13 | 0.311 |
| Sunflower     | 6.61<sup>c</sup> | 9.34<sup>c</sup> | 0.54 | 0.034 |
| Sesame        | 6.75<sup>c</sup> | 7.15<sup>c</sup> | 0.20 | 0.220 |
| Soybean       | 6.74 | 8.10<sup>c</sup> | 0.33 | 0.014 |
| Rice bran     | 6.65 | 7.36<sup>c</sup> | 0.18 | 0.192 |
| SEM           | 0.06 | 0.85 |     |       |
| p value       | 0.772 | 0.026 |     |       |
| Linoleic acid (cis-9, cis-12 C18:2) |       |       |       |
| Mustard       | 8.49 | 7.69 | 0.29 | 0.506 |
| Groundnut     | 8.45 | 7.86 | 0.34 | 0.457 |
| Sunflower     | 8.45 | 9.85 | 0.54 | 0.366 |
| Sesame        | 8.50 | 7.66 | 0.24 | 0.234 |
| Soybean       | 8.40 | 9.29 | 0.34 | 0.207 |
| Rice bran     | 8.97 | 8.20 | 0.12 | 0.580 |
| SEM           | 0.22 | 0.90 |     |       |
| p value       | 0.790 | 0.403 |     |       |
| CLA (cis-9, trans-11 C18:2) |       |       |       |
| Mustard       | 0.31 | 0.35<sup>c</sup> | 0.01 | 0.154 |
| Groundnut     | 0.31 | 0.34<sup>c</sup> | 0.01 | 0.137 |
| Sunflower     | 0.32<sup>c</sup> | 0.44<sup>c</sup> | 0.02 | 0.026 |
| Sesame        | 0.31 | 0.35<sup>c</sup> | 0.01 | 0.330 |
| Soybean       | 0.31<sup>c</sup> | 0.41<sup>c</sup> | 0.02 | 0.019 |
| Rice bran     | 0.31 | 0.36<sup>c</sup> | 0.02 | 0.204 |
| SEM           | 0.02 | 0.01 |     |       |
| p value       | 0.885 | 0.039 |     |       |

<sup>a,b</sup>Means with different superscript letters in a row differ significantly (p<0.05). <sup>c</sup>Means with different superscript letters in a column within a fatty acid differ significantly (p<0.05). SEM=Standard error of mean, CLA=Conjugated linoleic acid, VA=Vaccenic acid, FA=Fatty acid to achieve a significant effect on VA and cis-9, trans-11 CLA concentrations in rumen fluid in vitro. In vivo studies in lambs, heifers and goats have reported the enhancement of the CLA content in muscle and adipose tissue with addition of vegetable oils or seeds (safflower oil added up to 6% of the diet DM [27]; sunflower and linseed oils each at about 2.82% of diet DM [28]; sunflower and soybean oils each at 4.5% of the diet DM [29]). In lactating goats, the concentration of CLA in milk increased with safflower and linseed oil supplementation at 5% of diet [30]. Rice bran oil added in the concentrate mixture up to 6% linearly increased cis-9, trans-11 CLA and total CLA in milk of dairy cows [31]. Dai et al. [32] reported that the inclusion of vegetable oils (rapeseed, peanut, and sunflower seed oils each added at 2% of diet DM) increased the concentration of cis-9, trans-11 CLA. Increased VA and CLA concentrations in rumen fluid are attributed to partial biohydrogenation of linoleic acid and linolenic acid by ruminal microorganisms in response to oil supplementation [25]. Despite increases in concentrations of cis-9, trans-11 CLA and trans-11 C18:1 FA, the concentrations of C18:0 FA were not changed. The reason is unknown but it may be due to short duration of incubation. Again, concentrations of cis-9, trans-11 CLA were not significantly increased by sesame oil containing 39.6% C18:2 FA though soybean oil containing 45.8% C18:2 FA enhanced cis-9, trans-11 CLA in milk of cows [26]. Dai et al. [32] also, peanut oil supplementation containing 26.9% C18:2 FA of total FA of diet increased cis-9, trans-11 CLA in milk of cows compared with rapeseed oil containing 31.6% C18:2 FA of total FA in diet DM. With other in vitro studies, this in vitro study is useful to find out preliminary findings, which are required to be confirmed in long-term animal studies.

Conclusion

Supplementation of vegetable oils rich in linoleic acid and linolenic acid such as sunflower oil and...
soybean oil at a dose of 4% of the diet could greatly increase beneficial cis-9, trans-11 CLA and VA concentrations in rumen fluid. These healthy FA after absorption from the intestine may be enriched in milk and meat of ruminants, but this should be confirmed in vivo animal experiments.

**Authors’ Contributions**

GPM and AR carried out the experiment design. AR participated in practical work. AKP and GPM performed statistical analysis, data interpretation and writing of the manuscript. All authors read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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