The effect of vegetable and animal oils added to different forages and concentrates on the in vitro fermentation parameters in ruminants

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ABSTRACT
This study aimed to determine the effects of fermentation parameters of sunflower oil, vegetable oil blend, palm oil and chicken oil addition to sunflower meal, wheat grain, alfalfa herbage and corn silage at different rates (4% and 6%, dry matter basis). The oil supplementation up to 6% rate to the alfalfa herbage and corn silage did not adversely affect the level of in vitro gas production, metabolizable energy (ME), net energy lactation (NE_L), organic matter digestion (OMD) and molarities of acetic, propionic, butyric, iso-valeric, iso-butyric and valeric acids and number of total protozoa (P > 0.05). The oil supplementation to alfalfa herbage increased molarities of butyric, iso-valeric, iso-butyric and valeric acids and number of total protozoa (except sunflower oil) in rumen fluid (P < 0.05). Palm oil decreased the in vitro gas production, ME, NE_L and OMD values of sunflower meal (P < 0.05). Supplementation of vegetable oil blend to wheat grain increased ME, NE_L and OMD values and butyric acid molarity in rumen fluid (P < 0.05). The in vitro ruminal ammonia–nitrogen concentrations of alfalfa herbage and corn silage increased by vegetable oil blend, palm oil and chicken oil up to 6% (P < 0.05). As a result, the nutrient content of the feedstuffs used changed the in vitro ruminal fermentation values of the oil additive to be added. The addition of sunflower oil, vegetable oil blend, palm oil and chicken oil at 4% and 6% rates to corn silage, alfalfa herbage, sunflower meal and wheat grain feed found to differ on in vitro ruminal fermentation parameters.

Introduction
Dairy cow forage rations typically have low oil levels. The common forage roughage used in the rations contains approximately 1–3% in dry matter (DM). The physiological period in which the ration energy level should be increased the most in dairy cattle is the early lactation period (postpartum period or fresh period). At the beginning of lactation, it is necessary to increase the ration energy during this period so that dairy cattle do not enter into negative energy balance and reach the expected fertility values (number of inseminations per pregnancy, estrus at significant and regular intervals). Various vegetable and animal by-products containing distilled grains and animal oils (such as tallow, tallow, rendering oils, poultry slaughterhouse oils, fish oils) or oil (such as sunflower oil, corn oil) are used in the nutrition of ruminants (NRC 2001). When dietary oils enter the rumen, the ester bonds in phospholipids, glycolipids and triacylglycerols are hydrolysed (>85%) by ruminal lipolytic bacteria. The oil hydrolysis that takes place in the rumen is mediated by bacteria and they produce long and medium chain fatty acids, glycerol and galactose. Glycerol and galactose are fermented and converted to short chain fatty acids – volatile fatty acids (VFA) and CO2 and H2 gases (Jarvis and Moore 2010).

According to fatty acid profiles, increased dietary oil can affect the digestibility of feedstuff, ruminal protein breakdown and ruminal digestibility of nutrients consisting of non-structural carbohydrates (such as starch, pectin, disaccharides) and structural carbohydrates (such as cellulose, hemicellulose, non-starch polysaccharides) (Sterk 2011). In addition, problems such as negative effects on fermentation end products and other nutrients released after digestion can limit the use of high-energy oils (NRC 2001). The use of fat in cattle rations negatively affects ruminal digestion depending on the dose. It is also claimed that all other dietary fats, with the exception of medium chain saturated fatty acid (MCFA) supplementation, do not affect or even increase the total digestibility of NDF (Weld and Armentano 2017). Supplementation with soybean oil and rice bran oil at 3% rate dairy to goat diet was not affected by dry matter intake and fibre digestion but there were increased molarities of VFA of goat rumin fluid (Raval et al. 2021). Lunsin et al. (2012) demonstrated that supplementations of rice bran oil (including about 34% linoleic and 38% oleic acids) at 6% to diet of dairy cows at lactating decreased the digestion of NDF and DM and the concentration of acetate, but 2% and 4% supplementation did not change these values. In another study, Doreau and Ferlay (1995) have stated that vegetable oils in the feed have little or no effect on the ruminal ammonia–nitrogen concentration, and that microbial protein synthesis is mostly the result of the suppression of rumen microorganisms. Previous researchers stated that changing effect on the fibrolytic and amylolytic bacteria of
palm oil and vegetable oil was due to fatty acid profile (Dohme et al. 2001). Abubakr et al. (2014) reported that the addition of 5% palm oil to goat rations decreased the number of rumens bacteria Fibrobacter succinogenes, did not change the number of bacteria Ruminococcus flavefaciens and Ruminococcus albus, but decreased the number of ruminal methanogenic archaea. The previous study stated that oleic acid supplementations (0, 20, 40 and 60 mg/50 ml culture solution) at different percentages in the in vitro fermentation of grass species (Leymus chinensis) dose-dependent manner decreased in vitro methane production in goats. O’Brien et al. (2014) reported that the addition of oleic acid to grass silages reduced in vitro methane production. In studies examining the effects of oils on feedstuffs digestion in the rumen in vitro; in general, the effect of a single oil on the in vitro digestion of the feedstuffs mixture was investigated. The study investigating the effect of oils on roughage and concentrate feed is limited. This study hypothesizes that animal and vegetable oils with different fatty acid profiles will have different effects on the in vitro fermentation parameters of forages and concentrates with different nutrient matter profiles. This study aimed to determine the effects of fermentation parameters of sunflower oil, vegetable oil blend, palm oil and chicken oil addition to sunflower meal, wheat grain, alfalfa herbage and corn silage at different rates (4 and 6%) by the in vitro gas production technique.

Material and methods

The determination of fatty acid compositions in oil samples

Sunflower oil, vegetable oil mixture, palm oil and chicken oil, used in this study, were obtained from a feed factory from Konya Province, Turkey. The oil samples were stored sun-free aluminium packaged bottle with 1 L volume at +4°C until analysis. The oil samples were methylated with the three-stage modified procedure of Wang et al. (2015). The percentages of individual fatty acid methyl esters (FAME’s) in total fatty acid methyl esters were detected in a gas chromatograph with flame-ionization detection (GC-FID, Thermo Scientific, USA), which have automatic sampler device (Thermo Al 1310, USA). The GC-FID was studied with a FAME column (Thermo Scientific™ TRACE™, TR-FAME GC Columns, Catalog number: 260M153P, USA) and injection split temperature 255°C, column 140°C, and flow rate 30 ml/min processing method for 42 min. The FAME’s identification was performed by comparing the retention times with the expected retention times of standard mixture in chromatograms (Kara 2020).

The determination of chemical compositions in feed samples

In the study, alfalfa herbage (beginning of flowering) and corn silage were used as forage, sunflower meal and wheat grain were used as concentrated feed. The forage samples were dried in a thermostatically controlled cabinet (Lovidond, Switzerland) at 60°C for 48 h, and concentrated feeds were dried at 105°C for 24 h, and then dry matter (DM) contents of samples were calculated. Dried samples were milled in a grinder mill (IKA Werke, Germany) to a maximum particle size of 1 mm. Dry matter (DM), ash, crude protein (CP) (nitrogen x 6.25) and diethyl ether extract (EE) levels were determined according to the method reported by the AOAC (1995).

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analysed according to Van Soest et al. (1991). The non-fibrous carbohydrate (NFC) values were calculated according to NRC (2001). All analyses were carried out as triplicate.

The determination of in vitro total gas, methane and estimated digestion values

The in vitro ruminal fermentation values of the corn silages were analysed using an in vitro gas production technique. Rumen fluid, which will use in the in vitro gas production technique, was taken from two Brown Swiss-Simmental mix breed cattle. The 0.20 g DM of feedstuffs samples were incubated in 30 ml of the filtered rumen fluid plus buffer mixture (1:2, v/v) in an anaerobic glass fermenter with 100 ml volumes (Model Fortuna, Germany) (Menke et al. 1979). After 24 hours of incubation, the total gas volume was recorded from the calibrated scale in the in vitro glass fermenter. The metabolic energy (ME), net energy lactation (NE£) and organic matter digestion (OMD) values of samples were calculated using equations by Menke and Steingass (1988). The percentage of methane gas in the 24-hour total gas production was determined using an infrared methane analyser device (Sensors, Germany) (Kara et al. 2015). Each sample was studied in triplicate.

The determination of the patterns of in vitro fermentation fluid

The pH value of the fermentation fluid in the glass fermenter at 24 hours of the in vitro incubation was measured with a digital pH meter (Mettler Toledo, S220 pH/ion meter, Ohio, USA). Analysis of short-chain fatty acids (or volatile fatty acids – VFA’s) in the in vitro fermentation fluid was determined by using a gas chromatograph device (Thermo Trace 1300, Thermo Scientific, USA) with an autosampler (Thermo Al-1310, Thermo Scientific, USA). The GC device was equipped with a Flame Ionization Detector (FID), with polyethylene glycol columns (length: 60 m, i.d: 0.25 mm x 0.25 μm, film thickness: 0.25 μm) (TG-WAXMS, Thermo Scientific, USA) according to the study of Ershahince and Kara (2017). The number of total ciliated protozoa was determined according to the reported method by Boyne et al. (1957). The ammonia–nitrogen concentration of the in vitro fermentation fluid was determined using a commercial assay procedure (Megazyme, K-AMIAR 02/20, Wicklow, Ireland).

Statistical analysis

Statistical analysis of the nutrient matter data obtained as a result of the study was performed using the SPSS 22.0 package program. The effect of oil type and oil addition level on the in vitro digestion variables of each feed ingredient was
determined using Multidirectional Analysis of Variance (ANOVA), Tukey Multiple Range Test, one of the Multiple Comparison Tests, was used to determine the materiality. The means were separated by Tukey’s multiple range test at $P < 0.05$.

Results

**Nutrient matter content of feedstuffs**

The nutrient matter contents of the feedstuffs are given in Table 1. The highest CP content was in sunflower meal. The ADFom was in wheat grain as the lowest value and in alfalfa herbage as the highest value. The alfalfa herbage, corn silage and sunflower meal were 34.98%, 43.51% and 40.23%, respectively. The highest hemicellulose content was in corn silage. The NFC content was in wheat grain as the highest and in sunflower meal as the lowest (Table 1).

**Fatty acid content of oils**

The highest fatty acid percentages in total fatty acids of sunflower oil were linoleic (48.5%), oleic (34.9%), palmitic (8.49%) and stearic (5.36%) acids. The highest molarities of fatty acids in the vegetable oil mixture were linoleic (43.03%), oleic (26.26%) and palmitic (23.08%) acids. The highest molarities of fatty acids were palm oil palmitic (67.37%) and oleic (29.87%) acids. The highest fatty acids in chicken oil were oleic (29.79%), palmitic (28.46%), linoleic (18.51%) and stearic (16.59%) acids (Table 2). The percentages of MUFA and PUFA in total fatty acids of sunflower oil were determined as 35.59% and 50.18%, respectively. The percentages of MUFA and PUFA in total fatty acids of palm oil were determined as 29.31% and 0.34%, respectively. The molarities of MUFA and PUFA in total fatty acids of oil were found to be 38.27% and 18.77%, respectively. The percentages of MUFA and PUFA in total fatty acids in chicken oil were 27.66% and 43.70%, respectively (Table 2).

**In vitro total gas-methane production and ruminal estimated values**

The addition of sunflower oil to alfalfa herbage increased methane production ($P < 0.05$). The addition of vegetable oil mixture, chicken oil and palm oil at both oil levels, increased methane production ($P < 0.05$) (Table 3). In the evaluation of all oils, it was determined that the addition of 4% and 6% of different oils to alfalfa herbage has no effect on 24 hours in vitro gas production (ml/0.2 g DM), rumen fluid pH value, ME, NE$_L$ and OMd values ($P > 0.05$) (Table 3). The molarities of butyric, propionic, acetic and TVFA acids in the in vitro fermentation fluid. The addition of vegetable oil mixture to wheat grain increased in vitro ruminal total gas, ME, OMd and NEL parameters compared to those of the addition of chicken oil ($P < 0.05$) (Table 4).

The addition of 4% and 6% vegetable oil to sunflower meal decreased in vitro gas production, ME and NE$_L$ values ($P < 0.05$). The addition of 4% and 6% chicken and palm oils to sunflower meal increased the ruminal pH value ($P < 0.05$). In the evaluation of all oils, 4% and 6% addition of different oils to sunflower meal decreased in vitro gas production, methane gas production, ME and NEL values; increased the pH value ($P < 0.05$) (Table 5).

The addition of vegetable oil at 4% and 6% rates to blend wheat grain increased in the in vitro gas production, ME, OMd and NE$_L$ values ($P < 0.05$) (Table 6). The average effect of different oils at 4% and 6% rates to wheat grain decreased pH value of in vitro fermentation fluid. The addition of vegetable oil mixture to wheat grain increased in vitro ruminal total gas, ME, OMd and NEL parameters compared to those of the addition of chicken oil ($P < 0.05$) (Table 6).

**Variables of in vitro ruminal fermentation fluid**

The addition of vegetable oils and chicken oil to alfalfa herbage increased the molarities of valeric, iso-butyric, iso-valeric, butyric, propionic, acetic and TVFA acids, ammonia–nitrogen concentration and number of total ciliate protozoa in the in vitro fermentation fluid ($P < 0.05$). The molarities of butyric, acetic and TVFA acids in the in vitro fermentation fluid of oil supplementation (average of all oils) increased in vitro ruminal methane production of alfalfa herbage ($P < 0.05$). The methane production of alfalfa herbage supplemented palm oil was lower than those of alfalfa herbage supplemented other oils ($P < 0.05$) (Table 3).

The addition of 4% and 6% chicken oil to corn silage increased the ruminal pH of in vitro fermentation fluid ($P < 0.05$). In the evaluation of all oils, adding 4% and 6% different oils to corn silage in all oils did not affect in vitro total gas and methane productions, ME, NE$_L$ and OMd values ($P > 0.05$). For all oils, in vitro ruminal methane production increased at both oil levels; the pH value increased in all oils with 6% oil level ($P < 0.05$) (Table 4).

The addition of 4% and 6% palm oil to sunflower meal decreased in vitro gas production, ME and NE$_L$ values ($P < 0.05$). The addition of 4% and 6% chicken and palm oils to sunflower meal increased the ruminal pH value ($P < 0.05$). In the evaluation of all oils, 4% and 6% addition of different oils to sunflower meal decreased in vitro gas production, methane gas production, ME and NEL values; increased the pH value ($P < 0.05$) (Table 5).

The addition of vegetable oil at 4% and 6% rates to blend wheat grain increased in the in vitro gas production, ME, OMd and NE$_L$ values ($P < 0.05$) (Table 6). The average effect of different oils at 4% and 6% rates to wheat grain decreased pH value of in vitro fermentation fluid. The addition of vegetable oil mixture to wheat grain increased in vitro ruminal total gas, ME, OMd and NEL parameters compared to those of the addition of chicken oil ($P < 0.05$) (Table 6).

| CP | EE | Ash | ADFom | aNDFom | HemS | NFC |
|----|----|-----|-------|--------|------|-----|
| Alfalfa herbage | 15.40 | 1.45 | 9.95 | 30.14 | 34.98 | 4.86 | 38.22 |
| Corn silage | 3.61 | 0.89 | 10.45 | 28.06 | 43.51 | 15.46 | 41.55 |
| Sunflower meal | 31.30 | 2.31 | 6.35 | 28.46 | 40.23 | 11.78 | 19.81 |
| Wheat grain | 11.72 | 1.62 | 18.81 | 3.59 | 15.63 | 12.06 | 69.22 |

CP: crude protein, EE: ether extract, ADFom: detergent fiber detected by α-amylase and without ash, aNDFom: neutral detergent fiber detected by α-amylase and without ash, HemS: hemicellulose, NFC: non-fiber carbohydrate.
alfalfa herbage with palm oil and chicken oil were higher than those of alfalfa herbage with sunflower oil and vegetable oil mixture \((P < 0.05)\) (Table 7).

The addition of oil to corn silage increased the molarities of acetic, propionic, butyric and TVFA acids and ammonia–nitrogen concentration in the in vitro fermentation fluid \((P < 0.05)\). The total ciliated protozoa number of in vitro fermentation fluid of corn silage decreased with the increasing rate of oil addition \((P < 0.05)\). The number of total ciliated protozoa in the fermentation fluid of corn silages supplemented vegetable oils was lower than that of supplemented chicken oil \((P < 0.05)\) (Table 8).

The molarities of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids and TVFA in the fermentation fluid of sunflower meal supplemented vegetable oils added were lower than those of sunflower meal supplemented chicken oil \((P < 0.05)\). The addition of sunflower oil, palm oil and vegetable oil mixture at 4% and 6% rates to sunflower meal reduced the molarities of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids and TVFA in the fermentation fluid \((P < 0.05)\). The addition of oils up to 6% rate to

### Table 3. In vitro fermentation values of alfalfa herbage.

| Oil addition % | Total gas | Methane | pH | ME | OMd | NE |
|---------------|-----------|---------|----|----|-----|----|
| Sunflower oil |           |         |    |    |     |    |
| 0             | 44.49     | 7.34    | 6.61 | 9.12 | 67.84 | 5.25 |
| 4             | 48.65     | 10.03   | 6.33 | 9.69 | 71.54 | 5.73 |
| 6             | 52.16     | 11.66   | 6.45 | 10.17 | 74.65 | 6.13 |
| Vegetable oil blend | |         |    |    |     |    |
| 0             | 44.49     | 7.34    | 6.61 | 9.12 | 67.84 | 5.25 |
| 4             | 47.64     | 9.20    | 6.48 | 9.55 | 70.63 | 5.61 |
| 6             | 50.90     | 11.41   | 6.45 | 10.00 | 73.53 | 5.99 |
| Palm oil      |           |         |    |    |     |    |
| 0             | 44.49     | 7.34    | 6.61 | 9.12 | 67.84 | 5.25 |
| 4             | 48.71     | 9.46    | 6.46 | 9.70 | 71.58 | 5.73 |
| 6             | 50.90     | 11.41   | 6.45 | 10.00 | 73.53 | 5.99 |
| Chicken oil   |           |         |    |    |     |    |
| 0             | 44.49     | 7.34    | 6.61 | 9.12 | 67.84 | 5.25 |
| 4             | 42.81     | 9.85    | 6.46 | 8.90 | 66.34 | 5.05 |
| 6             | 43.81     | 10.47   | 6.45 | 9.03 | 67.24 | 5.17 |
| Oil level     |           |         |    |    |     |    |
| 0             | 44.49     | 7.34    | 6.61 | 9.12 | 67.84 | 5.25 |
| 4             | 46.95     | 10.16   | 6.43 | 9.29 | 68.94 | 5.39 |
| 6             | 45.73     | 10.16   | 6.46 | 9.29 | 68.94 | 5.39 |
| Oil type      | Sunflower oil | 48.43 | 9.68a | 6.46 | 9.66 | 71.34 | 5.70 |
| Vegetable oil blend | 47.67 | 9.32ab | 6.51 | 9.56 | 70.67 | 5.62 |
| Palm oil      | 43.08     | 7.97b   | 6.52 | 8.93 | 66.59 | 5.09 |
| Chicken oil   | 43.70     | 9.22ab  | 6.51 | 9.02 | 67.14 | 5.16 |

**P value**

- Oil type: 0.234 0.040 0.966 0.234 0.234 0.234
- Oil level: 0.652 <0.001 0.216 0.651 0.651 0.651
- Interaction: 0.259 0.037 0.998 0.259 0.259 0.259

**Total gas:** In vitro gas production as ml for 0.2 g DM at 24 h ruminal incubation; **methane:** In vitro methane production as ml for 0.2 g DM at 24 h ruminal incubation; **ME:** metabolic energy calculated from in vitro total gas production, as MJ/kg DM; **OMd:** organic matter digestion, calculated from in total gas production at 24 h ruminal incubation. **NEL:** net energy for lactation calculated from in vitro total gas production, as MJ/kg DM; a, b: The differences between the average values indicated by different letters for the silage type are important.

### Table 4. In vitro fermentation values of corn silage.

| Oil addition % | Total gas | Methane, ml | pH | ME | OMd | NE |
|---------------|-----------|-------------|----|----|-----|----|
| Sunflower oil |           |             |    |    |     |    |
| 0             | 49.88     | 8.90        | 6.30 | 9.19 | 66.19 | 5.13 |
| 4             | 43.78     | 9.11        | 6.30 | 8.36 | 60.77 | 4.43 |
| 6             | 50.02     | 11.45       | 6.33 | 10.02 | 71.64 | 5.83 |
| Vegetable oil blend | |         |    |    |     |    |
| 0             | 49.88     | 8.90        | 6.30 | 9.19 | 66.19 | 5.13 |
| 4             | 58.01     | 11.71       | 6.29 | 10.29 | 73.41 | 6.06 |
| 6             | 51.09     | 9.57        | 6.29 | 9.35 | 67.27 | 5.27 |
| Palm oil      |           |             |    |    |     |    |
| 0             | 49.88     | 8.90        | 6.30 | 9.19 | 66.19 | 5.13 |
| 4             | 53.27     | 9.73        | 6.31 | 9.65 | 69.20 | 5.52 |
| 6             | 52.42     | 9.71        | 6.37 | 9.53 | 68.45 | 5.42 |
| Chicken oil   |           |             |    |    |     |    |
| 0             | 49.88     | 8.90        | 6.30 | 9.19 | 66.19 | 5.13 |
| 4             | 49.11     | 9.55        | 6.32 | 9.08 | 65.50 | 5.04 |
| 6             | 48.26     | 9.40        | 6.38 | 8.96 | 64.74 | 4.94 |
| Oil level     |           |             |    |    |     |    |
| 0             | 49.88     | 8.90        | 6.30 | 9.19 | 66.19 | 5.13 |
| 4             | 51.04ab   | 10.02ab     | 6.30 | 9.34 | 67.22 | 5.26 |
| 6             | 51.95ab   | 10.03ab     | 6.34 | 9.47 | 68.02 | 5.36 |
| Oil type      | Sunflower oil | 49.89 | 9.82 | 6.31ab | 9.19 | 66.20 | 5.13 |
| Vegetable oil blend | 52.99 | 10.66 | 6.29b | 9.61 | 68.96 | 5.49 |
| Palm oil      | 51.86     | 9.44        | 6.33a | 9.45 | 67.95 | 5.35 |
| Chicken oil   | 49.08     | 9.28        | 6.33a | 9.08 | 65.48 | 5.04 |

**P value**

- Oil type: 0.209 0.360 0.012 0.209 0.209 0.209
- Oil level: 0.479 0.022 0.002 0.479 0.479 0.479
- Interaction: 0.033 0.053 0.090 0.033 0.033 0.033

**Total gas:** In vitro gas production as ml for 0.2 g DM at 24 h ruminal incubation; **methane:** In vitro methane production as ml for 0.2 g DM at 24 h ruminal incubation; **ME:** metabolic energy calculated from in vitro total gas production, as MJ/kg DM; **OMd:** organic matter digestion, calculated from in total gas production at 24 h ruminal incubation. **NEL:** net energy for lactation calculated from in vitro total gas production, as MJ/kg DM; a, b: The differences between the average values indicated by different letters for the silage type are important.
sunflower meal reduced the number of total ciliated protozoa in the fermentation fluid ($P < 0.05$). The addition of oil at 4% rate to sunflower meal increased the ammonia–nitrogen concentration in the fermentation fluid, but there was no difference with the addition of oil at 6% rate (Table 9).

The molarities of iso-butyric, iso-valeric, acetic and TVFA acids in the in vitro fermentation fluid of wheat grain supplemented palm oil were lower than those of wheat grain supplemented sunflower oil and chicken oil ($P < 0.05$). The number of total ciliated protozoa in the fermentation fluid of wheat grain supplemented vegetable oil mixture was higher than those of wheat grain supplemented chicken oil ($P < 0.05$) (Table 10).

**Discussion**

**Fatty acid composition**

The fatty acid profile of oils varies according to the raw material from which they are obtained, as well as being of vegetable and animal origin (NRC 2001). In the present study, the percentages of linoleic acid (48.5%), oleic acid (34.9%), palmitic acid (8.49%) and stearic acid (5.36%) in total fatty acids of sunflower oil were similar to the results of previous studies (Akkaya 2018; Çolak et al. 2020). Unlike the results of the present study, Rego et al. (2005) reported that sunflower oil contained 59.8% linoleic acid, 25.4% oleic acid, 3.5% stearic acid and 4.8% palmitic acid in total fatty acids. Besides, 13 different sunflower genotypes contained 49.9–55.7% linoleic acid, 29.9–37.8% oleic acid, 3.5–5.9% stearic acid and 5.0–7.9% palmitic acid in total fatty acids reported by Öztürk (2021). The vegetable oil mixture used in the present study included 43.03% linoleic, 26.26% oleic and 23.08% palmitic acids in the total fatty acids suggested that this oil mixture might be a blend of rapeseed, palm and soybean oils (Rego et al. 2005; Jokic et al. 2013; Mancini et al. 2015; Chew 2020; Öztürk 2021). Orsava et al. (2015) stated that 14 vegetable oil mixture comprised 2–79% linoleic acid, 6–71% oleic acid and 5–20% palmitic acid. The highest fatty acids of palm oil were palmitic acid (51.6%), oleic acid (23.2%), stearic acid (16.8%) and elaidic acid (5.6%). Mancini et al. (2015) found that palm oil contained 44.0% palmitic acid, 40% oleic acid, 10% linoleic acid and 4.5% stearic acid. Although there was a great deal of similarity between the fatty acid contents of the vegetable oils used in the study and the values reported in the literature, there are some minor differences. This situation can be attributed to the differences in the purity degree of the vegetable oil, the genotype of the plant, the environmental conditions (such as irrigation, soil type, stress factor) and the oil extraction method.

Chicken meat has about 30% oil content. Therefore, poultry slaughterhouse by-products (oil, skin and tissues) have a large oil content. In addition, it has an economic value, which is used in the production of biodiesel and can be an oil additive in animal feeds (farm animals and pet animals) (Mege et al. 2006; Marulanda et al. 2010; Lin and Tsai 2015). The chicken oil used in the study contained 33% oleic, 22% palmitic, 18% linoleic and 16% stearic acids, which was similar to the findings of the previous study (Arnaud et al. 2004; Marulanda et al. 2010). In addition, Peña-Saldarriaga et al. (2020) detected that chicken oil contained 36.9% oleic, 23.9% palmitic, 22.8% linoleic and 5.8% stearic acids is in total fatty acids. The difference between the chicken oil used in the study and the chicken oil in the literature in terms of some fatty acids may be due to the type of poultry abattoir products used, conditions of extraction method used and storage time of oils.

**In vitro ruminal fermentation**

In vitro ruminal gas production varies according to the levels and digestibilities of easily fermented carbohydrate and fibre.
substances in the feedstuffs. Forages are expected to have lower gas production than concentrates (especially starch-rich ones) in rumen. Oilseed meal is poor in starch, and ruminal gas production is expected to change according to the digestions of fibre substances (Menke 1988; NRC 2001). In the present study, the in vitro fermentation values (total gas production, ME, NEL, OMd) of alfalfa herbage, corn silage and wheat grain were not adversely affected by the addition of oil (4% and 6% sunflower oil, palm oil, vegetable oil mixture and chicken oil) demonstrated that oils do not adversely affect the microorganisms (producing cellulolytic and hemi-cellulololytic enzymes) (Bacteriodota and Firmicutes) that ferment this forage with high NDF content (Delgado et al. 2019). In the present study, the molarities of volatile fatty acids in the in vitro fermentation fluid of alfalfa herbage were not adversely affected by the oils used supports the in vitro gas production value, ME and OMd results. A previous study stated that the supplementation of palm oil at 3.5% to feedstuffs did not affect ruminal nutrient digestion (Grummer 1991). In the present study, the in vitro total gas and methane productions, ME and NEL values of sunflower meal (including high CP and high aNDFom) decreased by four different oils (sunflower, vegetable blend, palm and chicken oils) supplementation up to 6% rate. These decreases reached 20–30% with the increase in oil

### Table 6. In vitro fermentation values of wheat grain.

| Oil addition % | Total gas | Methane | pH | ME | OMd | NEa |
|----------------|-----------|---------|----|----|-----|-----|
| Sunflower oil  |           |         |    |    |     |     |
| 0              | 75.01     | 15.51   | 6.31| 13.06| 82.20| 902 |
| 4              | 78.59     | 17.21   | 6.26| 13.55| 85.39| 9.43 |
| 6              | 78.96     | 16.82   | 6.24| 13.60| 85.71| 9.48 |
| Vegetable oil blend |        |         |    |    |     |     |
| 0              | 75.01     | 15.51   | 6.31| 13.06| 82.20| 9.02 |
| 4              | 83.85     | 17.20   | 6.23| 14.27| 90.06| 10.04|
| 6              | 86.57     | 17.25   | 6.24| 14.64| 92.48| 10.35|
| Palm oil       |           |         |    |    |     |     |
| 0              | 75.01     | 15.51   | 6.31| 13.06| 82.20| 9.02 |
| 4              | 78.34     | 17.42   | 6.25| 13.52| 85.16| 9.40 |
| 6              | 83.53     | 18.21   | 6.29| 14.22| 89.78| 10.00|
| Chicken oil    |           |         |    |    |     |     |
| 0              | 75.01     | 15.51   | 6.31| 13.06| 82.20| 9.02 |
| 4              | 88.49     | 14.36   | 6.20| 12.18| 76.41| 8.27 |
| 6              | 75.16     | 13.25   | 6.26| 13.08| 82.34| 9.04 |
| Oil level      |           |         |    |    |     |     |
| 0              | 75.01     | 15.51   | 6.31a| 13.06| 82.20| 9.02 |
| 4              | 77.31     | 16.55   | 6.23b| 13.38| 84.26| 9.29 |
| 6              | 81.05     | 16.38   | 6.25b| 13.89| 87.58| 9.72 |

Total gas: in vitro gas production as ml for 0.2 g DM at 24 h ruminal incubation; methane: in vitro methane production as ml for 0.2 g DM at 24 h ruminal incubation; ME: Metabolic energy calculated from in vitro total gas production, as MJ/kg DM; NEL: net energy for lactation calculated from in vitro total gas production, as MJ/kg DM; OMd: organic matter digestion, calculated from in vitro total gas production at 24 h ruminal incubation. The difference between the average values indicated by different letters for the silage type is important.

### Table 7. Volatile fatty acids, ammonia-N and number of ciliated protozoa in the in vitro fermentation fluid of alfalfa herbage.

| Oil addition % | VA | IBA | IVA | BA | PA | AA | TVFA | NH3-N, g/L | Total ciliate protozoa ×10^5/ml |
|----------------|----|-----|-----|----|----|----|------|-----------|--------------------------------|
| Sunflower oil  | 0  | 0.88| 1.00| 1.82| 5.46| 7.97| 36.96| 54.98     | 16.60                          | 0.81 |
| 4              | 1.13| 1.27| 2.39| 6.89| 10.50| 47.75| 70.88| 20.55     | 0.79                          |
| 6              | 1.31| 1.44| 2.90| 7.59| 10.65| 35.26| 59.67| 22.30     | 0.83                          |
| Vegetable oil blend |    | 0.88| 1.00| 1.82| 5.46| 7.97| 36.96| 54.98     | 16.60                          | 0.81 |
| 4              | 1.13| 1.28| 2.51| 6.74| 9.64  | 34.03| 56.16| 19.35     | 0.99                          |
| 6              | 1.28| 1.34| 2.70| 7.33| 10.63| 43.05| 72.05| 22.40     | 1.18                          |
| Palm oil       | 0  | 0.88| 1.00| 1.82| 5.46| 7.97| 36.96| 54.98     | 16.60                          | 0.81 |
| 4              | 1.13| 1.27| 2.79| 8.03| 12.79| 58.78| 86.18| 19.45     | 0.92                          |
| 6              | 1.28| 1.35| 2.42| 6.84| 10.19| 48.14| 70.94| 19.70     | 1.01                          |
| Chicken oil    | 0  | 0.88| 1.00| 1.82| 5.46| 7.97| 36.96| 54.98     | 16.60                          | 0.81 |
| 4              | 1.13| 1.27| 2.47| 6.74| 9.64  | 34.03| 56.16| 19.35     | 0.99                          |
| 6              | 1.28| 1.34| 2.70| 7.33| 10.63| 43.05| 72.05| 22.40     | 1.18                          |
| Oil level      | 0  | 0.88| 1.00| 1.82| 5.46| 7.97| 36.96| 54.98     | 16.60                          | 0.81 |
| 4              | 1.13| 1.27| 2.79| 8.03| 12.79| 58.78| 86.18| 19.45     | 0.92                          |
| 6              | 1.28| 1.35| 2.42| 6.84| 10.19| 48.14| 70.94| 19.70     | 1.01                          |

AA, acetic acid; BA, butyric acid; PA, propionic acid; VA, valeric acid; IBA, iso-butyric acid; IVA, iso-valeric acid; TVFA, total volatile fatty acids (short chain fatty acids).
supplementation. It was observed that the nutrient content of feedstuffs and the used oils was effective on the in vitro gas production values.

Gas production in the in vitro gas production technique, nutrient digestion, ammonia production is also an indicator of protein breakdown (Menke 1988; Kara 2021). According to the results of the study, in vitro gas production values were harmony with changing in ammonia–nitrogen concentration and molarities (acetic, butyric and propionic acids) of volatile fatty acids in fermentation fluid, which was incubated alfalfa herbage. The oil (sunflower oil, vegetable oil blend, palm oil and chicken oil) supplementations to alfalfa herbage and wheat grain up to 6% rate positively affected the molarities of volatile fatty acids (acetic, propionic, butyric, valeric, iso-butyric and iso-valeric acids) in the rumen fermentation fluid was also determined. The use of oils up to 6% had no negative effect on alfalfa herbage, corn silage and wheat grain with high NFC and moderate CP contents. The decrease in the in vitro gas production values of sunflower meal with the addition of oil was paralleled with the decrease in molarities of straight (acetic, propionic, butyric and valeric acids) branched (iso-butyric and iso-valeric acids) short-chain fatty acids of fermentation fluid of sunflower meal. It can be thought that the increase in the molarities of these short-chain fatty acids with branched (iso-butyric and iso-valeric acids) and straight (acetic, propionic, butyric and valeric acids) chains of fermentation fluid of alfalfa herbage with oil supplementation (especially palm and chicken oil) increased the relative abundant of microorganisms in the rumen microbiome that use ammonia-nitrogen as protein degradation intermediates. The increase in the branched iso-acids (iso-butyric and iso-valeric acids) are also an indicator of ruminal protein degradation, and the increase in corn silage and alfalfa herbage with all oil supplementation and in wheat grain with palm oil and vegetable oil mixture supplementation indicated that they are formed as protein degradation intermediates. The increase in the

**Table 8. Volatile fatty acids, ammonia-N and number of ciliated protozoa in the in vitro fermentation fluid of corn silage.**

| Oil level | Oil type | Oil addition % | mmol/L, in vitro fermentation fluid | NH₃-N, g/L | Total ciliate protozoa ×10⁵/ml |
|-----------|----------|----------------|-------------------------------------|-----------|-----------------------------|
| 0         | Sunflower oil | 0              | 1.29 1.34 2.68 9.83                  | 15.17     | 60.18 91.48 94.58           |
| 4         | Vegetable oil blend | 1.38 1.37 2.78 10.12 | 15.64 62.76 95.03          | 109.63   | 0.86                       |
| 6         | Palm oil | 1.31 1.35 2.75 10.73                  | 16.39 64.66 98.24           | 121.74   | 0.77                       |
| 0         | Chicken oil | 1.29 1.34 2.68 9.83                  | 15.17 60.18 91.48           | 94.58    | 1.08                       |
| 4         | Vegetable oil blend | 1.38 1.40 2.86 10.58 | 16.56 65.51 99.28           | 117.63   | 0.61                       |
| 6         | Palm oil | 1.42 1.38 2.74 10.47                  | 17.00 65.11 99.11           | 110.44   | 0.71                       |
| 0         | Vegetable oil blend | 1.38 1.44 2.89 11.07 | 17.74 67.00 102.70          | 108.85   | 0.94                       |
| 4         | Vegetable oil blend | 1.31 1.35 2.65 10.36 | 16.40 63.38 96.38           | 113.81   | 0.97                       |
| 6         | Palm oil | 1.47 1.48 3.00 10.85                  | 17.11 66.09 101.09          | 85.26    | 1.06                       |
| 0         | Sunflower oil | 1.32 1.35 2.74 10.22                  | 15.73 62.53 94.91           | 108.65   | 0.90b                      |
| 4         | Vegetable oil blend | 1.36 1.37 2.76 10.48 | 16.67a 64.8a 98.36a          | 112.56a  | 0.93ab                     |
| 6         | Palm oil | 1.34 1.37 2.59 10.14                  | 16.74 63.48 96.46           | 114.12   | 1.33                       |
| 0         | Chicken oil | 1.47 1.48 3.00 10.85                  | 17.11 66.09 101.09          | 85.26    | 1.06                       |
| 4         | Vegetable oil blend | 1.38 1.39 2.78 10.60 | 16.72 64.81 98.70a          | 107.81a  | 0.88b                      |
| 6         | Palm oil | 1.38 1.39 2.78 10.60                  | 16.72 64.81 98.70a          | 107.81a  | 0.88b                      |
| 0         | Vegetable oil blend | 0.854 0.947 0.995 0.894 | 0.180 0.753 0.701          | 0.131    | 0.005                      |
| 4         | Vegetable oil blend | 0.066 0.367 0.517 0.011 | <0.001 <0.001 0.001         | 0.002    | 0.032                      |
| 6         | Vegetable oil blend | 0.191 0.445 0.461 0.400 | 0.153 0.362 0.299          | 0.040    | 0.040                      |

AA, acetic acid; BA, butyric acid; PA, propionic acid; VA, valeric acid; IBA, iso-butyric acid; IVA, iso-valeric acid; TVFA, total volatile fatty acids (short chain fatty acids).
butyric acids) in the fermentation liquid of sunflower meal. However, for sunflower meal and wheat grain, the change of the molarities of iso-acids in the rumen fluid with the oil addition changed negatively with the ammonia ratio in the rumen fluid. The decrease in the in vitro gas and estimated digestion values of sunflower meal with the supplementations of four different oils were compatible with the decrease in molarities of the straight and branched short-chain fatty acids and the total number of protozoa in the in vitro fermentation fluid. Considering the effectiveness of oils in reducing the molarity of branched-chain iso-acids (iso-valeric and iso-butyric acids) in the fermentation liquid of sunflower meal, palm oil reduced iso-acids more seriously. The branched-chain iso-acids are mainly built up from the degradation products of the amino acids valine, isoleucine, leucine and proline and should in turn be used for the biosynthesis of those amino acids and higher branched chain volatile fatty acids (Apajalatti et al. 2019). It can be said that the ruminal breakdown of some amino acids was adversely affected by the addition of oil in the study of sunflower meal with a ruminal by-pass value of approximately 90% (NRC 2001). It was an expected situation that there was a relationship between the NFC content of the feedstuffs used in the study and the pH value and TVFA molarities in the in vitro fermentation fluid. The wheat grain, which has the highest TVFA molarity (about 109–123 mmol/L rumen fluid) and the highest NFC

Table 9. Volatile fatty acids, ammonia-N and number of ciliated protozoa in the in vitro fermentation fluid of sunflower meal.

| Oil type         | VA (mmol/L) | IBA | IVA | VA | PA | BA | AA | TVFA | NH3-N, g/L | Total ciliated protozoa × 10⁵/ml |
|------------------|-------------|-----|-----|----|----|----|----|------|-----------|-------------------------------|
| Sunflower oil    | 2.17        | 2.12| 4.15| 9.75| 15.32| 61.88| 96.23| 80.55| 0.95      |                               |
| Vegetable oil blend | 2.17       | 2.12| 4.15| 9.75| 15.32| 61.88| 96.23| 80.55| 0.95      |                               |
| Palm oil         | 2.17        | 2.12| 4.15| 9.75| 15.32| 61.88| 96.23| 80.55| 0.95      |                               |
| Chicken oil      | 2.17        | 2.12| 4.15| 9.75| 15.32| 61.88| 96.23| 80.55| 0.95      |                               |
| Oil level        | 2.17        | 2.12| 4.15| 9.75| 15.32| 61.88| 96.23| 80.55| 0.95      |                               |
| Oil type         | Sunflower oil | 2.02 | 2.01 | 4.01 | 9.29 | 14.80 | 57.48 | 88.17 | 0.69      |                               |
| Vegetable oil blend | 2.02       | 2.01| 4.01| 9.29| 14.80| 57.48| 88.17| 0.69 | 0.69      |                               |
| Palm oil         | 2.02        | 2.01| 4.01| 9.29| 14.80| 57.48| 88.17| 0.69 | 0.69      |                               |

Table 10. Volatile fatty acids, ammonia-N and number of ciliated protozoa in the in vitro fermentation fluid of wheat grain.

| Oil type         | VA (mmol/L) | IBA | IVA | VA | PA | BA | AA | TVFA | NH3-N, g/L | Total ciliated protozoa × 10⁵/ml |
|------------------|-------------|-----|-----|----|----|----|----|------|-----------|-------------------------------|
| Sunflower oil    | 1.93        | 1.53| 3.12| 15.15| 15.00| 67.15| 104.87| 86.89| 1.43      |                               |
| Vegetable oil blend | 1.93       | 1.53| 3.12| 15.15| 15.00| 67.15| 104.87| 86.89| 1.43      |                               |
| Palm oil         | 1.93        | 1.53| 3.12| 15.15| 15.00| 67.15| 104.87| 86.89| 1.43      |                               |
| Chicken oil      | 1.93        | 1.53| 3.12| 15.15| 15.00| 67.15| 104.87| 86.89| 1.43      |                               |
| Oil level        | 1.93        | 1.53| 3.12| 15.15| 15.00| 67.15| 104.87| 86.89| 1.43      |                               |
| Oil type         | Sunflower oil | 2.01 | 1.76 | 3.20| 8.87| 14.96| 59.64| 92.08| 0.77      |                               |
| Vegetable oil blend | 2.01       | 1.76| 3.20| 8.87| 14.96| 59.64| 92.08| 0.77 | 0.77      |                               |
| Palm oil         | 2.01        | 1.76| 3.20| 8.87| 14.96| 59.64| 92.08| 0.77 | 0.77      |                               |

AA, acetic acid; BA, butyric acid; PA, propionic acid; VA, valeric acid; IBA, iso-butyric acid; IVA, iso-valeric acid; TVFA, total volatile fatty acids (short chain fatty acids).
content (69.22% DM) reached the lowest acidity (pH 6.25–6.28) of ruminal fermentation fluid.

The effect of vegetable oil blend and palm oil addition (average of 4% and 6%) to all short-chain fatty acids molarities in the fluid of the in vitro fermentation of sunflower meal was lower than the addition of chicken oil, has been suggested that this situation is related to the fatty acid profile (palmitic acid, linoleic acid, stearic acid) in its content (Dohme et al. 2001; Fiorentini et al. 2015; Pi et al. 2019). The addition of vegetable oil mixture to wheat grain feed increased in vitro gas production, OMd and NEL values. In the effect of oil types on the in vitro fermentation of wheat grain feeds, it is seen that the vegetable oil mixture has higher in vitro gas production, ME, OMd and NEL values than chicken oil. In terms of these values, the fermentation values of the vegetable oil blend in wheat grain feed were similar to sunflower oil and palm oil were determined. In the presented study, individual additions of each oil to wheat grain feed had no effect on the pH value of the in vitro fermentation fluid. However, at the average effects of oil addition levels, it was seen that the pH value of the fermentation fluid of wheat grain feed decreased with the addition of 4% and 6% oil.

**Methane production**

Methane, which is released as a result of enteric fermentation of consumed feed materials, means loss of feed energy in ruminants. The release of methane, the energy that needs to be used in metabolism, results in wastage of feed materials, but also leads to environmental pollution and global warming (Gastelen et al. 2015; Gang et al. 2020). To compare the ruminal methane production of feeds, studies have been intensified in recent years and the antimethanogenic activities of feeds containing high levels of secondary (such as tannin, saponin) metabolites have been emphasized (Chaves et al. 2006; Hassanat et al. 2014; Kara et al. 2015; Niu et al. 2021). The dose-dependent increase in methane production by oil additives to alfalfa herbage and corn silage may be related to an increase in the rate of acetic acid in the rumen or an increase in the number of methanogenic archaea. *Euryarchaeota* (mainly *Methanobrevibacter* and *Methanospirillum* genus in *Methanobacteriales* order) and *Thermoplasmata* (*Methanomassiliicoccales* order) orders in microbiome of ruminous fluid have been characterized as hydrogenotrophic, utilizing H₂/CO₂ or H₂/methanol produced by various fermentative bacteria in the anaerobic degradation of feedstuffs, especially forages (Poulsen et al. 2013). The hydrogeneotrophic pathway catalysing the conversion of CO₂ to methane is dominant in the rumen and occurs in *Methano- brevibacter* spp (Danielsson et al. 2017). The oil supplementation (sunflower oil, vegetable blend oil and chicken oil) up to 6% rates increased the *in vitro* methane production of corn silage and alfalfa herbage may be due to these oils increased the *in vitro* anaerobic breakdown (gas production) of forage and the molarity of acetic acid (precursor of methane), and these oils do not hurt methanogenic archaea. Abubakr et al. (2014) reported that the addition of 5% palm oil to goat rations decreased the number of rumens bacteria *Fibrobacter succinogenes*, did not change the number of bacteria *Ruminococcus flavefaciens* and *Ruminococcus albus*, but decreased the number of ruminal methanogenic archaea. In the present study, the effect of oil types on the *in vitro* ruminal methane production differed according to the feedstuff material used. The effect of palm oil on the *in vitro* methane production was evident in alfalfa herbage and sunflower meal. The anti-methanogenic effect for alfalfa herbage (6% rate) and sunflower meal (4 and 6% rates) of palm oil may be due to the high percentage of palmitic acid (approximately 67%) was similar to previous studies (Dohme et al. 2001; Chantaprasarn and Wanapat 2008; Beauchemin et al. 2009; O’Brien et al. 2014). Previous study stated that oleic acid supplantations (0, 20, 40 and 60 mg/50 ml culture solution) at different percentages on the *in vitro* fermentation of grass species (*Leymus chinensis*) dose-dependent manner decreased *in vitro* methane production in goats. O’Brien et al. (2014) reported that the addition of oleic acid to grass silages reduced *in vitro* methane production. Ding et al. reported that 12 g/day coconut oil (with high palmitic acid) supplementation to *Tibetan* sheep rations reduced methane production by 61.3%. In another study, Dohme et al. (2001) reported that the addition of 5% palmitic acid to the total diet did not change *in vitro* methane production. In a study (Beauchemin et al., 2009), was determined that using crushed sunflower, flaxseed and canola seeds increased the energy of the diet causing an average of 13% decrease in ruminal methane production. The lowering effect on methane production of palm oil may be related to the high content of palmitic acid (67.4%) and oleic acid (29.9%) (O’Brien et al. 2014; Wu et al. 2016). This result may be due to the higher content of myristic acid in chicken oil. Machmüller (2006) reported significant reductions in methane production (up to 50%) with the addition of both lauric acid and myristic acid to the diet at levels below 3%. Odongo et al. (2007) reported a 36% reduction in methane production in dairy cows fed a diet supplemented with 5% myristic acid. However, other reports indicate that myristic acid does not affect rumen methanogenesis (Dohme et al. 2001; Soliva et al. 2003).

Study results and previous study results were compared, ad it was thought that the difference between oil supplementation and ruminal ammonia nitrogen concentration might be related to variables such as substrate used (ration, forage, concentrate), fatty acid content of the used oil, *in vitro* method used, content of the rumen fluid used (such as the number of bacteria, protozoa, archaea, pH value), composition of the ration consumed by the donor animal. Oils rich in saturated fatty acids or unsaturated fatty acids used as dietary oil supplements may limit the number of ruminal protozoa both in vivo and *in vitro*. Among the C18 fatty acids, linoleic acid has been shown to have the most pronounced toxic effect on protozoan populations (Ivan et al. 2001; Baah et al. 2007; Dayani et al. 2007). A study with barley silage-based ration and sunflower seed oil (6%) showed that *Iotricha* (the *Iotricha* and *Dasytricha* spp.) and cellulytic ciliates (*Polyplastron*, *Diplodinium* and *Enoploplastron*) was more sensitive to linoleic acid than *Entodinium* (Ivan et al. 2001). In contrast, Várádyová et al. (2008) showed that the addition of 5% sunflower oil to the hay and barley diet reduced only
the populations of Isotricha spp in the sheep rumen. In the presented study, the number of ciliated protozoa in the \textit{in vitro} fermentation fluid increased with the addition of 6% oil (average effect of four different oils) to alfalfa herbage and corn silage and decreased with the addition of 4% and 6% oil (average effect of four different oils) to sunflower meal. Chicken oil supplementation to wheat grain had a negative effect on the number of ruminal protozoa compared to the vegetable oil blend. According to the results of the study reported by Belanche et al. (2015), that is similar to the findings of the presented study, it shows parallelism with the number and type of ciliated protozoa and the change in the molarity of TVFA in the rumen fluid. It has been understood that the effect of fatty acids on the number of ruminal protozoa varies according to the nutrient content of the used feedstuff and the type of ciliate protozoa changed (Ivan et al. 2001; Baah et al. 2007; Dayani et al. 2007; Váradyová et al. 2008).

As a result, the nutrient content of the feedstuff changes the \textit{in vitro} ruminal fermentation values of the oil additive to be added. Adding 4% and 6% sunflower oil, vegetable oil mixture, palm oil and chicken oil to alfalfa herbage and corn silage did not affect \textit{in vitro} digestion values. The addition of sunflower oil, vegetable oil mixture, palm oil and chicken oil, alfalfa herbage and corn silage increased \textit{in vitro} ruminal methane emission. The supplementation of 4% and 6% sunflower oil, vegetable oil mixture and chicken oil additions to sunflower meal did not adversely affect \textit{in vitro} digestion values. The addition of sunflower oil, vegetable oil mixture, palm oil and chicken oil to sunflower meal linearly reduced \textit{in vitro} methane emission and total protozoa count. Sunflower oil, palm oil and chicken oil supplementation up to 6% to wheat grain did not adversely affect \textit{in vitro} digestion values. \textit{In vitro} digestion values of wheat grain feed increased linearly with vegetable oil blend. \textit{In vitro} rumen fluid ammonia nitrogen concentration increased with the addition of 4% and 6% vegetable oil mixture, palm oil and chicken oil to alfalfa herbage and corn silage; ammonia nitrogen concentration \textit{in vitro} rumen fluid of wheat grain feed and sunflower meal did not change with the addition of four different oil sources was concluded.

In addition to the difference in fatty acid profiles of the oils, \textit{in vitro} ruminal fermentation values differ according to the level of nutrients and anti-nutritional factors of the nutrient materials used; in this case, in the future, it is necessary that the effects of fatty acids on ruminal digestion and digestive end products of feed should be investigated by \textit{in vivo} studies.

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No potential conflict of interest was reported by the author(s).

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**Data availability statement**

The authors declare that data supporting the study findings are also available to the corresponding author.

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**References**

Abubakr A, Alimon AR, Yaakub H, Abdullah N, Ivan M. 2014. Effect of feeding palm oil by-products based diets on total bacteria, cellulosolytic bacteria and methanogenic archaea in the rumen of goats. PLoS One. 9(4):e95713.

Akay M. 2018. Fatty acid compositions of sunflowers (Helianthus annuus L.) grown in east Mediterranean region. Rivista Ital Del Sost Grasse. 95:239–247.

AOAC. 1995. Association of Official Analytical Chemists. Washington (DC): Association of Official Analytical Chemists.

Apajalahti J, Viennola K, Raatikainen K, Holder V, Moran CA. 2019. Conversion of branched-chain amino acids to corresponding isoacids - an in vitro tool for estimating ruminal protein degradability. Front Vet Sci. 6.311. doi:10.3389/fvets.2019.00311.

Arnaud E, Relkin P, Pina M, Collignon A. 2004. Characterisation of chicken fatty acid profile in milk of chickens fed a vegetable oil blend. Eur J Lipid Sci Technol. 106:391–598. doi:10.1002/ejlt.200400946.

Baah J, Ivan M, Hristov MN, Koenig KM, Rode LM, McAllister TA. 2007. Effects of potential dietary antiprotozoal supplements on rumen fermentation and digestibility in heifers. Anim Feed Sci Technol. 137(1–2):126-137. doi:10.1016/j.anifeedsci.2006.11.004

Beauchemin KA, McGinn SM, Benchaar C, Holtshausen L. 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. J Dairy Sci. 92(S):2118–2127.

Belanche A, de la Fuente G, Newbold CJ. 2015. Effect of progressive inoculation of Fauna-Free sheep with Holotrichia protozoa and Total-Fauna on rumen fermentation, microbial diversity and methane emissions. FEMS Microbiol Ecol 362:1–10.

Boyne AW, Eadie JM, Raitt K. 1957. The development and testing of a method of counting rumen ciliate protozoa. J Gen Microbiol. 17:414–423. doi:10.1002/21287-17.2-414.

Chapatrasarn N, Wanapat M. 2008. Effects of sunflower oil supplementation in cassava hay based-diets for lactating dairy cows. Asian-Aust J Anim Sci. 21:42–50. doi:10.5713/ajas.2008.60421.

Chaves AV, Thompson LC, Iwaasa AD, Scott SL, Olson ME, Benchaar C, Veira DB. 2015. Economic and environmental comparison of cold-pressed rapeseed (Brassica napus L.) oil: chemistry and functionality. Food Res Int. 131:108997. doi:10.1016/j.foodres.2020.108997.

Çolak Ç, Hasançebi S, Kaya Y. 2020. Determination of high oleic acid property in sunflower oil by using molecular markers. Anadolu J AARI. 30:57–68. doi:10.18615/anadolu.727207.

Danielsson R, Dicksved J, Sun L, Gonda H, Müller B, Schnürer A, Bertilsson J. 2017. Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. Front Microbiol. 8:226. doi:10.3389/fmicb.2017.00226.

Dayani O, Ghorbani GR, Alikhani M, Rahmani HR, Mir PS. 2007. Effects of dietary whole cottonseed and crude protein level on rumen protozoal population and fermentation parameters. Small Rum Res. 69:36–45. doi:10.1016/j.smallrumres.2005.12.007.

Delgado B, Bach A, Guasch I, González C, Elcoso G, Pryce JE, Gonzalez-Rejano O. 2019. Whole rumen metagenome sequencing allows classifying and predicting feed efficiency and intake levels in cattle. Sci Rep. 9:11. doi:10.1038/s41598-018-36673-w.
Sterk AR. 2011. Ruminal fatty acid metabolism altering rumen biohydrogenation to improve milk fatty acid profile of dairy cows. Wageningen University the Netherlands, 188. [accessed: 02.01.2022]. https://edepot.wur.nl/178582.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Váradyová Z, Kšídayová S, Siroka P, Jalč D. 2008. Fatty acid profiles of rumen fluid from sheep fed diets supplemented with various oils and effect on the rumen ciliate population. Czech J Anim Sci. 52:399–406. doi:10.17221/2322-CJAS.

Wang J, Wu W, Wang X, Wang M, Wu F. 2015. An effective GC method for the determination of the fatty acid composition in silkworm pupae oil using a two-step methylation process. J Serbian Chem Soc. 80:9–20. doi:10.2298/JSC140401073W.

Weld KA, Armentano LE. 2017. The effects of adding fat to diets of lactating dairy cows on total-tract neutral detergent fiber digestibility: a meta-analysis. J Dairy Sci. 100:1766–1779. doi:10.3168/jds.2016-11500.

Wu D, Xu L, Tang S, Guan L, He Z, Guan Y, Tan Z, Han X, Zhou C, Kang J, Wang M. 2016. Influence of oleic acid on rumen fermentation and fatty acid formation in vitro. PLoS One. 11(6):e0156835. doi:10.1371/journal.pone.0156835.