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RESEARCH ARTICLE

The Effect of Some Vegetable Oils Added to Dairy Calf Rations on *In Vitro* Feed Value and Enteric Methane Production

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ABSTRACT

The aim of this study was to determine the effects of the addition of Safflower, Sunflower and Corn vegetable oils to dairy cattle rations on *in vitro* gas and methane production, true dry matter (TDMD), organic matter (TOMD) and NDF (TNDFD) digestibilities values and microbial protein (MP) production. Dairy cattle TMR ration consisting of milk feed, corn silage, alfalfa hay and meadow hay was prepared as the control group, and the experimental groups were prepared with the addition of safflower, sunflower and corn vegetable oils at the level of 3% in each of the control groups, respectively. Vegetable oils added to the diet significantly affected *in vitro* gas production and organic matter digestibility (OMD). Methane (ml) production values in the experimental groups varied between 10.00 and 10.71 ml. The Metabolic energy (ME) and OMD values of the control and experimental groups varied between 7.00 and 7.29 MJ/kg DM and between 53.78 and 51.20. TDMD values of the rations were determined between 48.49 and 52.63%. While the control group had the highest TDMD value, the ration containing safflower oil had the lowest TDMD value. TNDFS values of the rations varied between 67.26 and 68.80%. As a result; Since the vegetable oils added to the ration increase the net energy lactation (NE_L) content of the ration, it can be said that it used to meet the energy needs of high milk yielding cattle in the lactation period, provided that they do not exceed the upper limits specified in the literature.

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Introduction

Ruminants play a key role in sustainable agriculture by using the roughage and agricultural by-products that are not consumed by humans thanks to the microorganisms in the digestive system, and by converting them into high-quality foods such as meat and milk that can be consumed by human beings (Wright & Klieve, 2011; Gerber et al., 2013). In the rumen, volatile fatty acids are converted into H_2 and CO_2 as a result of fermentation of microorganisms (Hegarty & Gerdes, 1998). Archaea play an important role in rumen health and in producing and removing enteric methane from the rumen by hydrogenotrophic pathway by using H_2, which has toxic effects for certain microorganisms, as a substrate together with CO2 (Hook et al., 2010). About 90% of the methane produced by archaea in ruminants is burped and the remainder is expelled from the rectum (Murray et al., 1976). It has been reported that methane formed in ruminant animals causes energy loss due to the removal of metabolic hydrogen and carbon produced by fermentation (Martinez-Fernandez et al., 2014). They reported that the energy loss due to methane in ruminants is 2-12% of the digestible energy, and this rate rises to 15-18% in the case of feeding with low quality roughage (Kaya et al., 2012). On the other hand, it was stated that the greenhouse gas effect of methane released from ruminants as a result of fermentation is the effective gas after CO_2 gas (Sejian et al., 2011). For this reason, they stated that there are various management strategies in reducing the methane produced by ruminants, such as the quality of the roughage in the ration, organic acids, phenolic-containing plants and feed intake level, the speed of the feed passing through the digestive tract, the saturation level of the fat in the ration (Kaya et al., 2012; Gomaa et al., 2018). Martin et al. (2010) reported that the most effective method to reduce methane is

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to supplement the diets of ruminants with fat. Mathison (1997) stated that the addition of 4% canola oil to the ration containing 85% concentrated feed reduced enteric methane by 33%, in addition, coconut oil was the most suppressive of fiber digestibility in the rumen. The reduced methane production due to the addition of unsaturated fats to the diet has been attributed to the fact that fatty acids can serve as electron acceptors during biohydrogenation in the rumen (McAllister et al., 1996). If enteric CH_4 production is reduced, substrates (H and CO_2) can be included in the fermentation products, which will allow the animal to increase its energy use efficiency (Haisan et al., 2014). In this study, it was investigated whether the addition of 3% sunflower, safflower and corn oil to the diets of dairy cattle had an effect on gas production, methane production, microbial protein and *in vitro* true digestibilities in order to reduce methane released as a result of fermentation of feeds in ruminants.

Materials and Methods

Feed Material and Treatment Groups

In the study, TMR rations (Control) consisting of 25% milk feed, 25% corn silage, 15% alfalfa hay and 35% dry meadow grass given to dairy cattle in Atatürk University Research and Application Farm were used as feed material. Experimental groups were prepared by adding 3% safflower, sunflower and corn oils to TMR rations.

Chemical Analyzes

The samples belonging to the experimental groups were ground to pass through a 1 mm sieve for chemical analysis and dry matter, ether extract, crude fiber, crude protein, crude ash contents were determined according to the methods reported by AOAC (1998). The ADF, NDF and ADL contents of the ration content were determined with the ANKOM 2000 Fiber Analyzer device according to the method reported by Van Soest et al. (1991). The nutrient contents of the experimental groups are given in Table 1 on the basis of dry matter.

Table 1. Chemical compositions of the experimental groups

Ingredients (g/kg DM)	Control	Safflower Oil	Sunflower Oil	Corn Oil
Corn Silage	250	250	250	250
Alfalfa Hay	150	150	150	150
Dry Meadow Grass	350	350	350	350
Milk Feed (21% HP)	250	250	250	250
Safflower Oil	-	30	-	-
Sunflower Oil	-	-	30	-
Corn Oil	-	-	-	30
Chemical Composition (%)				
DM	92.23	92.53	92.53	92.53
CP	11.93	11.93	11.93	11.93
EE	4.52	7.52	7.52	7.52
NDF	59.12	59.12	59.12	59.12
ADF	30.78	30.78	30.78	30.78
ADL	14.06	14.06	14.06	14.06
CF	20.89	20.89	20.89	20.89
CA	7.73	7.73	7.73	7.73

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, CF: Crude fiber, CA: Crude ash

Determination of In Vitro Gas and Methane Production and Microbial Protein Amounts

The amount of gas released as a result of 24-hour incubation of the ration by method (GP) Menke et al. (1979) according to the technique reported. The methane contents produced at the end of the 24-hour incubation were determined using an Infrared methane analyzer (Goel et al., 2008). In order to determine the dry matter content of the ration content fermented with rumen fluid at the end of the incubation, the residue in glass syringes was boiled with NDF solution in a 250 ml glass beaker and filtered by passing through gooch por 1 crucibles (Blümmel et al., 1997). The ME, NE_L and OMD values of the ration content were found with the following equations stated by Menke and Steingass (1988). CP, EE, CA data used in the equations are used as %.

$$ME = 2.2 + 0.1357 \times GP + 0.057 \times CP + 0,002859 \times EE$$

$$NE_L = 0.101 \times GP + 0.051 \times CP + 0.112 \times EE$$

$$OMD = 14.88 + 0,8893 \times GP + 0.448 \times CP + 0.651 \times CA$$

TDMD, PF, Microbial protein production (MP) and Microbial protein production efficiency (MPPE) values of ration content Blümmel et al. (1997) 's calculated using the formulas reported below.

TDMD = Dry matter incubated (mg) – Remaining dry matter (mg)

PF = (TDMD / Gas Production)

MP = (TDMD – (2.2 x Gas Production)

MPPE = ((TDMD – (2.2 x Gas Production))/ TDMD)

Determination of In Vitro True Dry Matter, NDF and Organic Matter Digestibilities

Ankom Daisy II incubator D 220 device was used to determine the in vitro true nutrient digestibility of the ration contents by the filter bag method (Van Soest et al., 1991). The rumen fluid used in the study was obtained from the rumen of 3 healthy head rams of Awassi breed, who were 2 years old and between 60-70 kg body weight, who received slaughter approval from the ethics committee of Erzurum Meat and Fish Institution, as reported by Kılıç and Abdiwali (2016). was brought to Atatürk University Faculty of Agriculture, Animal Science Department, Feed Analysis Laboratory in a screw cap bottle with a capped thermos container containing water at approximately 39°C. Rumen fluid was used for *in vitro* true nutrient digestibilities after filtering through four-layer cheesecloth by providing anaerobic environment under CO_2 gas. It was prepared to contain a total of 2 L of buffered rumen fluid, accompanied by the addition of CO_2 gas (1600 ml buffer solution + 400 ml rumen fluid) in each glass jar in the Daisy II incubator device. It was incubated in the Daisy II incubator for 48 hours. After 48 hours, all the bags were taken out of the glass jars and kept under tap water until clear water flowed, and after drying in the open, they were kept in the pre-prepared oven at 105 ºC for 2-3 hours. After weighing the bags removed from the oven, the *In vitro* true dry matter digestibility (IVTDMD) was determined by applying the following formula. In order to determine the actual NDF content of the ration content, the bags, which were removed and dried in an oven after 48 hours of incubation, were boiled in ANKOM 2000 Fiber Analyzer with NDF solution at 100°C for 75 minutes. After the process was finished, the bags were washed 2-3 times in water and dried in an oven at 105 ºC for 2-4 hours. *In vitro* true NDF digestibilities (TNDFD) was calculated by substituting the dried bags in the formula. In order to determine the *in vitro* true OM digestibilities (TOMD) of the ration content, the bags were burned at 550 ºC for 3-4 hours in the muffle furnace, and at the end of the incineration process, they were weighed on a precision scale and calculated with the following equation.

%IVTDMD=100 – (((D3-D1)/(D2-D1)*100)

%TNDFD=100 – (((D4-D1)/(D6-D7)*100)

%TOMD=100 – (((D5-D2)/(D8-D7)*100)

D1: Tare of F57 bags, D2: Dry weight of ration content, D3: Amount of ration remaining after incubation, D4: Content of ration treated in NDF solution in Ankom 200/220 cellulose analyzer and dried in an oven, D5: Amount remaining after burning in a 550 °C ash oven, D6: % NDF content of the ration content, D7: DM content of the ration content, D8: % organic matter content of the ration content

Statistical Analysis

Statistical analyzes of the data obtained from the research were made using the SPSS 26.0 package program. Duncan multiple comparison test (Duncan, 1955) was used to compare the means of the groups.

Results and Discussion

Table 2 shows the 24-hour in vitro gas and methane production values and the mean values of ME and NE_L contents of the groups formed by adding 3% sunflower, safflower and corn vegetable oils to dairy cattle TMR rations.

Table 2. Average *in vitro* gas and methane production and ME and NEL values of the experimental groups

Groups	Gas (ml)	Methane (ml)	Methane (%)	ME (MJ/kgDM)	NE_L (MJ/kgDM)
Control	80.17[a]	10.71	13.36	7.29[a]	4.35[b]
Safflower Oil	76.07[b]	10.68	14.04	7.17[a]	4.52[a]
Sunflower Oil	72.93[c]	10.00	13.72	7.00[b]	4.4[b]
Corn Oil	76.07[b]	10.02	13.19	7.17[a]	4.52[a]
SEM	2.06	0.62	1.14	0.01	0.00
P	0.000	0.430	0.685	0.002	0.002

a,b,c: Means shown with different letters in the same column are significantly different (P<0.05). SEM: Standard error means.

Gas production values of the rations differed according to the added oils, and the lowest was observed in the group containing 3% sunflower oil (72.93 ml) and the highest in the control group (80.17 ml). Similar to the current study, it was stated that the addition of oil to the ration (Vargas et al., 2017, 2020) decreased the gas production values compared to the control ration. It was determined that there was no significant difference between the groups in terms of methane production (P>0.05). Compared to the *in vitro* methane (ml) content of the control group, it was determined that the *in vitro* methane (ml) values produced in the rations containing sunflower and corn oil were numerically low. Compared to the % methane values obtained from the control group, a numerical decrease of 1.3% was observed in the corn oil-containing group. Since vegetable oils affect the amount of gas produced as a result of 24-hour incubation, differences in ME and NE_L values occurred. The highest ME value was determined in the control group, and the lowest value was determined in the group containing sunflower oil. The highest NE_L content was found in the rations containing safflower and corn oil, and

the lowest in the control group. The values determined for the NE_L contents of the rations were determined by Zhang et al. (2021) were found to be lower than the values reported. Among the groups prepared according to the classification made by Lopez et al. (2010), the groups with corn and sunflower added are partially antimethanogenic. It has been stated that the % methane content of feeds with low antimethanogenic potential should be between >11 and ≤14. It is stated that the fact that the rations have antimethanogenic properties is important in terms of energy use efficiency and environment in animal nutrition (Navarro-Villa et al., 2013).

True digestive dry matter (TDDM), OMD, PF, MP and MPPE values of the experimental groups are given in Table 3. When Table 3 was examined, it was determined that the vegetable oils added to the ration affected the OMD values (P<0.05).

Table 3. TDDM, PF, MP and MPPE values of the experimental groups

Groups	TDDM (mg)	PF (mg/ml)	MP (mg)	MPPE (%)	TDDM (%)
Control	319.96	3.86^{b}	137.36	42.92^{b}	53.78^{a}
Safflower Oil	315.36	4.01^{ab}	142.11	45.02^{ab}	52.32^{b}
Sunflower Oil	316.35	4.19^{a}	150.25	47.49^{a}	51.20^{c}
Corn Oil	317.04	4.03^{ab}	143.79	45.24^{ab}	52.32^{b}
SEM	9.76	0.18	10.58	2.42	0.26
P	0.937	0.042	0.419	0.047	0.000

a,b,c: Means shown with different letters in the same column are significantly different (P<0.05). SEM: Standard error means. TDDM: True digestive dry matter (mg), PF: Scale factor, MP: Microbial protein production (mg), MPPE: Microbial protein synthesis efficiency (%).

The highest OMS value was observed in the control group (53.78%), while the lowest was observed in the group containing sunflower oil (51.20%). Addition of vegetable oil to dairy cattle TMR rations increased PF value (P<0.05). The PF value was found to be the lowest with 3.86 mg/ml in the control group, and the highest value with 4.19 mg/ml in the group containing sunflower oil. It has been reported that the theoretical PF values of the feeds used in the nutrition of ruminant animals vary between 2.75 and 4.41 and these values should be taken into account in determining the synthesizing efficiency of microbial protein (Blümmel et al., 1997). It has been reported that the higher the PF value of a feed, the higher the microbial protein synthesis efficiency of that feed will be depending on it (Blümmel & Lebzien, 2001). It was determined that the TDDM values of the experimental groups varied between 315.36 and 319.96 mg, and the difference between the values was not significant (P>0.05). The MP values of the control and treatment groups were found to be between 137.36 and 150.25, and the difference was found to be statistically insignificant (P>0.05).

Average values obtained from control and treatment groups created by adding vegetable oil to dairy cattle TMR rations are given in Table 4.

Table 4. Effects of vegetable oils added to the ration on IVTDMD, TNDFD and TOMD

Groups	IVTDMD (%)	TNDFD (%)	TOMD (%)
Control	52.63	68.80	93.96
Safflower Oil	48.49	67.26	93.93
Sunflower Oil	48.56	67.74	94.01
Corn Oil	48.62	67.45	93.99
SEM	7.74	6.46	0.01
P	0.263	0.880	0.661

IVTDMD: *in vitro* true dry matter digestibility, TNDFD: *in vitro* true NDF digestibility, TOMD: *in vitro* true OM digestibility; SEM: Standard error means.

The differences between IVTDMD, TNDFD and TOMD values were not found significant *in vitro* conditions in the rations created with the addition of vegetable oil (P>0.05). Unlike the present study, Vargas et al. (2017) reported in their study that there was a decrease in IVTDMD in the group containing sunflower oil compared to the control rations. It was observed that there was a numerical decrease in the TNDFD values in the rations containing vegetable oil compared to the control ration. This is due to the fact that the crude fat content of the ration increases due to the addition of vegetable oil, which negatively affects cellulose and fiber digestion (Ayaşan & Karakozak, 2011). Similarly, it has been reported that fiber and cellulose digestion is suppressed in diets created by adding fat to the diet (Bayat et al., 2017; Beck, 2017; Darabighane et al., 2021).

Conclusion

Vegetable oils added to dairy cattle TMR rations significantly affected gas production and OMS values (P<0.05). The decrease in the *in vitro* NDF digestibility of the experimental groups is thought to be due to the decrease in gas production. As a result; It can be said that since the vegetable oils added to the ration increase the net energy lactation content of the ration, it can be used to provide the energy needs of high milk yielding cattle in the lactation period, provided that it does not exceed the upper limits specified in the literature, and by increasing the PF value, it will increase the microbial protein production and synthesis efficiency in the rumen.

Conflict of Interest

The authors declare that they have no conflict of interest.

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