ISTANBUL UNIVERSITY
CERRAHPAŞA

Addition of Medium-Chain Fatty Acids to Lamb Diets: Effects on Performance, Rumen Characteristics, Blood and Carcass Parameters

Dilek AKÇİN, Gülcan DEMİREL

Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa, İstanbul, Turkey

Cite this article as: Akçin, D., & Demirel, G. (2021). Addition of medium-chain fatty acids to lamb diets: Effects on performance, rumen characteristics, blood and carcass parameters. *Acta Veterinaria Eurasia*, *47*(3), 169–178.

ORCID iDs of the authors: D.A. 0000-0000-0000-0000; G.D. 0000-0002-6864-5134

Abstract

This experiment was carried out to investigate the effect of medium-chain fatty acid (MCFA) supplementation on feed intake, animal performance, rumen characteristics, blood lipid metabolites, and carcass parameters in Kivircik lambs. Lambs were allocated into three groups (eight lambs/treatment) and the experimental groups were as follows: Control, 10MCFA (1.0 g MCFA/kg feed) and 18MCFA (1.8 g MCFA/kg feed). On days 0, 28, and 56, lambs were weighed and rumen and blood samples were collected, and all lambs were slaughtered on day 56. According to the results obtained in the present study, animal performance was not affected by the dietary treatment ($p > .05$). There was no difference in rumen pH but the concentrations of rumen ammonia (on day 56) and propionic acid level (on days 28 and 56) were lower in 18 MCFA lambs measured at 3 hours after feeding ($p > .05$). Under the conditions and duration of this study, supplementing diets with MCFA did not affect lamb performance and carcass parameters but affected rumen fermentation parameters.

Keywords: Carcass traits, growth performance, lamb, medium-chain fatty acids

Introduction

Fats are added to ruminant feeds at different levels and in different forms to meet energy needs of high-yielding animals, increase feed utilization, and manipulate the digestion and absorption of other nutrients. Thus, it is possible to meet the energy needs of animals, and more importantly, to provide energy from carbohydrates in a more economical way due to their high energy source (Doreau & Chilliard, 1997). In addition, a number of studies aim to increase the amount of some fatty acids in the composition of meat and milk, which have been determined to have beneficial effects on human health (Demirel et al., 2004; Ferreira et al., 2014; Ponnampalam et al., 2006). Besides, their effects on reducing methane production have also been revealed (Bayat et al., 2017; Machmüller et al., 2000). In some cases, fats added to ruminant rations affected the live weight gain and feed utilization rates of animals at various levels, while in others, no effect was reported on these parameters. The reason for these contradictory results is attributed to factors affecting the rumen flora, such as the source and amount of fat and nutrient composition of the ration (Bhatt et al., 2011; Ghafari et al., 2016). The fats used in ruminant rations can cause a decrease in cellulosic and organic matter digestion, methane formation, ammonia concentration, and acetate:propionate ratio (Ghafari et al., 2016). Therefore, the most desirable sources of fat for use in rations are those characterized by minimal negative effects on rumen fermentation and high digestibility. However, it is difficult to provide these two features at the same time. Unsaturated fatty acids are highly digestible, but on the other hand, they reduce cellulose digestibility in the rumen as a result of an inhibitory effect on cellulolytic microorganisms. While saturated fatty acids affect the cellulose digestion less in the rumen, they still reduce the digestibility (Firkins & Eastridge, 1994; NRC, 2001). Coconut

Corresponding Author: Gülcan DEMİREL • **E-mail:** gdemirelstanbul.edu.tr

Received: March 3, 2021 • **Accepted:** June 3, 2021 • **Available Online Date:** September 6, 2021 • DOI: 10.5152/actavet.2021.21030

Available online at actavet.org

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

oil as a medium-chain fatty acid source was also studied in a few in vivo studies in lamb, and results showed that coconut oil directly inhibited rumen methanogenic archaea and may change their metabolic activity, as well as the composition of the rumen methanogenic population (Machmüller, 2006; Machmüller et al., 2000). Bhatt et al. (2011) also showed the effect of coconut oil on feed and nitrogen efficiency. Medium-chain fatty acids (MCFAs) have been studied more intensively in monogastric animals, and some of their properties have been revealed (Hermans et al., 2012; Hong et al., 2012). Studies on the effect of medium-chain fatty acids on lamb performance and methane production are few, and most of the studies on this area are about fats with triglyceride structure. However, in vivo studies on ruminants, especially lambs, are limited. In the current study, and the effects of the medium-chain fatty acid supplementation as free fatty acids in lamb rations containing different forage:concentrate ratios on live weight gain, feed intake, blood triglycerides, total cholesterol, HDL and LDL cholesterol levels, ammonia-nitrogen (NH_3-N) levels, rumen volatile fatty acid (VFA) levels, and some carcass characteristics were investigated. This study also aims to obtain an idea about a possible reduction of methane emission, which may lead to increased energy output and an increase in live weight.

Method

Animals, Diets, and Experimental Procedure

Twenty-four weaned male Kivircik lambs were used in the experiment. The animals were divided into three groups of eight lambs each. The experimental groups were as follows (Table 1): A control group without MCFA addition (50:50 forage:concentrate ratio); the 10 MCFA group with 1 g/kg (50:50 forage:concentrate ratio), and 18 the MCFA group with 1.80 g/kg (70:30 forage:concentrate ratio). Aromabiotic BFC, a source of MDFA that is added to lamb feed, was supplied from Vitamex (Booiebos 5 9031 Drongen, Belgium) and contains 60% MCFA (C6:0, C8:0, C10:0, C12:0) which are present in an activated free fatty acid form with silicon dioxide support. Chemical analysis showed that it contained 47% ether extract and 18.50% moisture.

The experiment lasted for 8 weeks following a one-week adaptation period. During the trial, the lambs were weighed at two-week intervals. The concentrates and grass hay allowance were based on their live weights and dry matter intake. The amount of concentrate feed and alfalfa hay given were estimated after weighing according to the average weight of the group of lambs, from data in Senel (1993), and water was available ad libitum. Blood samples were collected before the morning feed on the days 1, 28, and 56 of the experiment. Serum was separated by centrifugation (3000 g, 20 min) and stored at −18°C. The rumen contents were taken from lambs using a stomach tube, three hours after feeding on days 0, 28, and 56. The trial period lasted for 56 days and all the lambs were slaughtered at the end of the experiment.

Slaughter Procedures and Carcass Measurements

The animals were slaughtered in the abattoir at the University of İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine. For 12 hours before slaughter, the animals had access to water, but not to feed. Lambs were slaughtered after live weights were recorded and electrical stunning was performed. Hot carcass weight was recorded after removal of head, skin, feet, and internal organs (e.g., lungs, trachea, liver, heart, spleen, pancreas, gastrointestinal tract, diaphragm, and testicles). The hot carcass weight comprised kidneys and perinephric pelvic fat. Gastrointestinal tract content was excluded in determining empty body weight. Carcass pH levels were measured between the 12th and 13th thoracic vertebrae by digital pH meter (Testo 205, Testo AG, Germany) immediately after dressing (pH_0), and at 24 hours after slaughter (pH_{24}).

Chemical and Laboratory Analysis of Feeds and Samples

Moisture, ash, crude protein ($N \times 6.25$), and fat content of trial feeds were determined according to the standard methods of AOAC (2005), but neutral detergent fiber (NDF) fraction was determined using the procedure described by Van Soest et al. (1991), and acid detergent fiber (ADF) was determined using the method described by Goering and Van Soest (1970) as adapted for the Ankom200 Fiber Analyzer (Ankom Technology, USA).

Blood Metabolites and Ruminal Liquid Measurements

The samples were first kept at room temperature the day the blood analyses were performed, and measured using commercial kits (Clinical Analyzer, Tokyo Boeki Medical System, Niigata Mechatronic Co. Ltd., Japan) at the Central Laboratory of the Veterinary Faculty of Istanbul University-Cerrahpasa. Spinreact kits (Spinreact, Spain) were used for the analysis of total protein, albumin, triglyceride, cholesterol, HDL (High Density Lipoprotein), and LDL (Low Density Lipoprotein). Phosphorus (P) and Calcium (Ca) analysis was also performed using commercial kits (Cormay, Poland).

Total volatile fatty acids (VFA), and acetic, propionic, and butyric acids, NH_3-N concentrations, and pH were determined in rumen fluid. Samples were taken via stomach tube from all lambs three hours after the morning feed on days 0, 28, and 56 of the trial. Rumen pH was then immediately measured using a pH meter (Hanna Instruments, Romania). Rumen fluid samples were then strained through four layers cheesecloth. Further, 1 mL of 25% metaphosphoric acid was added to 5 mL of the homogenate. The samples were incubated for 30 minutes after mixing, centrifuged at 2000 rpm for 10 minutes, and 2 mL was taken from the supernatant and transferred to Eppendorf tubes. Until the day of analysis, samples in Eppendorf tubes were stored at −18°C in deep freeze. Levels of volatile fatty acids were determined by gas chromatography (Column 80/120 Carbopack B-DA/4% Carbowax 20M, carrier gas nitrogen 24 mL/min., column heat FID, 200°C). The NH_3-N concentration of ruminal liquid was also measured according to the method of Chaney and Marbach (1962).

Table 1

Ingredients and Chemical Composition of Feeds (%)

Ingredients	Control (50:50 F/C ratio)	10 MCFA (50:50 F/C ratio)	18 MCFA (30:70 F/C ratio)	Alfalfa hay
Barley	15.5	15.5	15.5	
DDGS	8.0	8.0	8.0	
Corn	11.0	11.0	11.0	
Wheat	7.0	7.0	7.0	
Wheat bran	14.3	14.3	14.3	
Bonkalit	11.0	11.0	11.0	
Soya bean meal	7.0	7.0	7.0	
Sunflower meal, 36–38%	10.0	10.0	10.0	
Sunflower meal, 28%	7.0	7.0	7.0	
Limestone	2.0	2.0	2.0	
Salt	.5	.5	.5	
Fat	.5	.4	.32	
Molasses	6.0	6.0	6.0	
MCFA	-	.10	.18	
Mineral[1], vitamin[2], premix	.15	.15	.15	
Total	100.0	100.0	100.0	
Composition (%)				
Dry matter	87.03	87.02	87.21	92.14
Crude protein	17.35	17.34	17.41	16.85
Crude fiber	5.05	5.10	5.06	37.2
NDF	21.49	21.68	20.67	46.16
ADF	7.81	7.33	7.51	33.92
Ether extract	4.13	4.09	4.46	2.10
Calcium	1.07	1.10	1.04	1.23
Phosphorus	.56	.56	.53	.26
ME, kcal/kg[3]	2773	2724	2715	2280

Note: [1]Mineral Premix: each kg contained 10,000 mg Cu, 50,000 mg Fe, 50,000 mg Mn, 50,000 mg Zn, 50 mg Co, 800 mg I, 150 mg Se, 340,000 mg Ca
[2]Vitamin Premix: each kg contained 66,700,000 IU Vitamin A, 16,700,000 IU Vitamin D3, 167,000 IU Vitamin E, 42,000 mg Vitamin B_1, 25,000 mg Vitamin B_2, 125 mg Vitamin B_{12}, 125,000 mg Niacin
[3]Calculated values (Alderman,1985; Sundstol,1993)
F/C = forage:concentrate.

Statistical Analysis

One-way analysis of variance and the Duncan test were applied in the SPSS package program to compare the animal performance and carcass characteristics of the lambs from the different experimental groups (Ozdamar, 2003). In the statistical analysis of blood and rumen fluid parameters, the General Linear Model-GLM procedure was applied, and as a "between-subject factor" in the statistical model; the measurement time was "within subject factor." Since the effect of binary integrations between factors is observed, interactions in the statistical model are also included. Statistical analyses were performed using the SPSS 13.0 statistical package (SPSS Inc., Chicago, Illinois, USA).

Results

The initial and final average live weights of lambs in each group are given in Table 2. At the end of the experiment, the final average live weights were 32.96, 31.01, and 31.92 kg for the control, 10MCFA, and 18MCFA groups, respectively. Significant difference was not found between the groups during all weighing periods ($p > .05$). Similarly, the average daily gain was not different among groups at the end of the study ($p > .05$; Table 2). Feeding with/without MCFA resulted in numerically similar feed intake and lower feed efficiency rates in the MCFA groups.

Table 2

Animal Performance and the Carcass Measurements of Lambs Fed With/Without MCFA

	Groups				
	Control (50:50 F/C ratio)	**10 MCFA (50:50 F/C ratio)**	**18 MCFA (30:70 F/C ratio)**	**SED**	***p***
Animal performance					
Average initial weight, kg	23.40	23.75	23.05	1.136	.972
Average final weight, kg	32.96	31.01	31.92	1.102	.931
Average daily gain, g	170.75	154.24	158.26	.007	.634
Daily feed intake, kg	1.43	1.43	1.41		
Daily DM intake, kg	1.28	1.28	1.27		
Feed conversion ratio	7.50	8.30	8.02		
Carcass measurement					
Hot carcass weight, kg	16.48	15.88	15.30	.631	.767
Cold carcass weight, kg	15.44	14.85	14.28	.586	.737
Hot dressing percentage	47.66	47.98	46.18	.507	.317
Cold dressing percentage	44.67	44.87	43.09	.474	.254
Carcass pH_0	6.36	6.60	6.60	.033	.908
Carcass pH_{24}	5.72	5.73	5.69	.021	.685
Organ weights, kg					
Liver	.59	.56	.55	.005	.847
Lungs and trachea	1.61	1.48	1.43	.013	.409
Spleen	.064[a]	.057[a]	.053[b]	.001	.035
Heart	.162	.159	.153	.005	.847
Testicle	.217	.223	.210	.017	.959
Tail fat	.24	.24	.18	.015	.162
Mesenteric fat	.41	.36	.47	.052	.701

Note: F/C = forage:concentrate.

Discussion, Conclusion and Recommendation

Researches on the effects of fatty acid sources in lamb diet on live weight and daily weight gains have shown mostly similar results. Coconut oil added at the level of 5% to lamb diets (Bozzolo et al., 1993) did not affect lamb performance during 20–40 and 40–60 days of the experiment. Bhatt et al. (2011) showed that 0, 25, 50, and 75 grams of coconut oil added per kilogram of feed in lamb diets had no effect on live weight and average live weight gain ($p > .05$). However, they reported a not statistically but numerically significant decrease in live weight gain when used at higher than 50 g/kg. One lamb study used forage to concentrate ratio of 25:75 (Dutta et al., 2008), and palm oil, which is very rich in MCFA, was added to lamb diets at different levels (0, 2.50%, 5%, 7.50%, 10%), and the difference between the groups for live weights was insignificant ($p > .05$). On the other hand, improved animal performance had also been reported when MCFA were used. For example, Jordan et al. (2006) found that coconut oil addition to heifer diets caused higher average live weight gain ($p < .05$). There are a number of studies which have reported that the use of coconut oil, a good source of MCFA, reduces dry matter consumption (Lovett et al., 2003; Machmüller & Kreuzer, 1999; Sutton et al., 1983). Bhatt et al. (2011) reported decreased dry matter intake in groups fed lamb feed supplemented with 75 g/kg coconut oil compared to groups supplemented with 0, 25, and 50 g/kg coconut oil. On the other hand, Jordan et al. (2006) showed that the addition of 250 grams of coconut oil (2.60%) daily did not change dry matter consumption in heifers. Bozzolo et al. (1993) reported significantly lower feed utilization rates in the experimental group of lambs after the first 20 days of feeding with 5% coconut oil, but they found similar rates in all groups through the rest of the trial. In both groups, they even reported sharp increases in feed utilization rates over the last 40–60 days of fattening, which was attributed to a reduction in digestive efficiency of around 24–26 kg live weight. Similar to these results, feed efficiency rates in the current study were 6.85, 7.42 and 7.09 for the control, 10 MCFA, and 18 MCFA groups respectively in the first 28 days of trial.

Table 3

Blood Metabolites of Kivircik Lambs Fed With/Without MCFA on Days 0, 28, and 56 (mg/dL)

	Groups				
	Control (50:50 F/C ratio)	**10 MCFA (50:50 F/C ratio)**	**18 MCFA (30:70 F/C ratio)**	**SED**	***p***
On day 0					
Cholesterol	31.00	26.50	33.03	1.224	.079
Triglycerides	4.62[a]	1.63[b]	4.50[a]	.778	.210
HDL	28.75[a]	23.50[b]	30.88[a]	1.268	.042
LDL	3.50	1.50	4.20	.478	.071
Ca	10.04[a]	9.39[b]	10.29 [a]	.131	.009
P	5.27	5.29	5.43	.162	.923
Albumin	2.86	2.86	2.90	.027	.829
Total Protein	5.65	5.70	5.72	.051	.846
On day 28					
Cholesterol	45.63	43.75	39.63	1.79	.392
Triglycerides	10.62	12.63	10.50	1.60	.843
HDL	36.38	36.88	37.50	.91	.889
LDL	7.13	9.88	9.38	.64	.174
Ca	9.40[c]	11.12[b]	12.89[a]	.19	.000
P	6.60	6.33	5.99	1.44	.228
Albumin	2.98	2.95	3.03	.29	.574
Total protein	5.87	5.82	5.81	.07	.925
On day 56					
Cholesterol	53.88[a]	52.13[a]	43.50[b]	1.729	.024
Triglycerides	4.62	7.75	10.63	1.173	.110
HDL	39.78	39.38	37.50	.907	.487
LDL	8.63	11.75	7.88	.722	.060
Ca	10.54	10.71	10.18	.136	.288
P	6.82	6.85	7.08	.160	.797
Albumin	3.18	3.15	3.10	.030	.604
Total protein	6.38	6.17	6.00	.086	.189

Note: [a,b,c]Significance level of differences between groups at the same sampling time.
F/C = forage:concentrate.

Data on the changes of blood metabolites are presented in Table 3. No difference was observed between the groups in the blood parameters on day 28, except for Ca level which was high in 18MCFA group fed a high alfalfa ratio. The level of cholesterol in the lambs from the 18MCFA group was lower compared to the control and the 10 MCFA group on day 56 ($p < .05$). Consistent with these results, in a study comparing the MCFA source with soybean oil (Yeom et al., 2003), there was no significant difference in triglyceride levels between groups, and lower cholesterol levels were observed in the 18MCFA group ($p < .001$). In contrast to the data reported in this study, HDL levels were significantly lower in the MCFA group in their study. Researchers have reported that saturated fats generally increase cholesterol, and that the cholesterol reduction observed in their work may be a situation unique to goats. The current study also suggests that the MCFA source reduces the cholesterol level in lambs, indicating that this condition is due to the MCFA source. Jambrenghi et al. (2007) and Muci et al. (1992) reported a 20% reduction in serum total cholesterol and a 32% reduction in cholesterol ester and triglyceride levels in lambs fed rations with flaxseed oil.

Total protein levels were within the normal reference range of 6–7.9 g/dL (Swenson, 1977). Albumin levels in healthy sheep vary rather widely, ranging from 2.4 mg/dL to 4 mg/dL

(Cripps et al., 1985; Van Zyl, 1967). In the current study, average blood albumin levels were found between 2.86 and 3.18 mg/dL for all treatment groups. Reference values for blood calcium for sheep were varied, ranging between 11.5 and 13 mg/dL (Swenson, 1977). Although calcium levels in this study were lower than reference values, these values are consistent with expected values for lambs of this age. However, in all treatment groups, blood phosphorus level was within reference ranges (4–7 mg/dL; Swenson, 1977). The analysis of albumin, total protein, calcium, and phosphorus in the blood, taken at three different periods in the study showed no significant difference among the groups and data suggest that there were no major metabolic problems in any treatment group.

Rumen fermentation parameters are presented in Table 4. In the current study, at the beginning of the experiment (day 0), the rumen pH of the control group was 6.03 and lower than the two experimental groups ($p < .05$). On the other hand, the pH values obtained at the middle (28th day) and at the end (56th day) of the study did not differ between the control and experimental groups ($p > .05$), and pH values were between 6.10 and 6.40. Similarly, Sondakh et al. (2012) reported that coconut meal at different levels as a methanogonic inhibitor did not have an effect on rumen pH ($p > .05$), although differences in ruminal propionic acid level were significantly different ($p < .05$). Feeding beef cattle two different fat levels (0% and 6%) with different roughage rates (10% and 30%) caused the rumen pH values to be in the range of 5.74–6.04, and the differences were not statistically significant (Zinn & Plascencia, 1996). Similarly, it has been reported that rumen pH from lambs fed diets supplemented with coconut oil at different levels (0, 25,50, 75 g/kg) were not different, and ranged from 6.31 to 6.63 (Bhatt et al., 2011). Bozzolo et al. (1993) also showed that coconut oil at 50 g/kg, similar to the above results, had no effect on the rumen pH level. When coconut oil was used at 7% in cattle diets, rumen pH did not differ from the control group ($p > .05$, Kongmun et al., 2011). On the other hand, it has been reported that the rumen pH was increased in Muzafarnagari lambs fed with palm oil (Dutta et al., 2008).

The study by Hristov et al. (2009) in dairy cattle showed different effects of chemical and physical structures of the fat on rumen metabolism. It was determined that cows fed diets with coconut oil (530 g/day) containing 45% lauric acid and 18% myristic acid had decreased rumen pH but diets supplemented with lauric (240 g/day) and stearic acid (240 g/day) did not cause a change in pH. Consistent with this work, the difference in pH between the groups may be due to the fact that the fatty acid in the source used is not in triglyceride form but in the form of free fatty acid.

The results of the NH_3-N levels in rumen liquids are presented in Table 4. There was no significant difference among groups at the beginning and on day 28 of the experiment. However, at the end of the experiment, the highest NH_3-N was observed in the control group (15.48 mg/dL), and the differences between the experimental groups were significant ($p < .01$). Studies on the effects fats and fatty acids on rumen NH_3-N in ruminants have produced conflicting results with significant increases or decreases (Gómez-Cortés et al., 2008). Hristov et al. (2009) reported that long-chain unsaturated fatty acids reduced the concentrations of sugars and soluble protein, which decreased the concentration of protozoa, ammonia, and VFA, while increasing the concentrations of free amino acids. NH_3-N levels were found to be low when the same researchers used coconut oil and lauric acid in dairy cattle diets ($p < .05$). These results were also consistent with results obtained from Jouany et al. (1988) who used a diet supplemented with palm, coconut, and canola oil.

At the beginning of the experiment, and on days 28 and 56, VFA analysis was performed in rumen liquids, and mean values for the groups are presented in Table 4. There were significant differences in VFA concentrations between experimental groups ($p < .05$). Many studies have been carried out with the addition of fats or various fatty acid sources to inhibit methane production in the rumen, and different VFA levels were reported. It was concluded that factors such as species, age, roughage:concentrate feed ratios, and the chemical properties of the oil supplemented influenced the results (Toprak, 2015). When the total VFA levels in the current study were examined (Table 4), numerically lower VFA concentrations in MCFA-supplemented groups were observed and the results suggested that rumen fermentation may be affected as a result of a decrease in the number of rumen protozoa. Additionally, acetic acid was detected at lower concentrations than in the control group, in the two experimental groups to which MCFA was added. The reduction in acetic acid by the addition of MCFA, on day 28 in the current study, may have been due to a certain level of inhibition of protozoa, with a 30% contribution to ruminant cellulose digestion (Demeyer, 1981). should be corrected as following; Machmuller et al. (2000) reported that protozoa numbers in sheep rumen fluid were affected by oil sources after feeding for seven weeks, and protozoa counts for control, crystalized fat, coconut, rape seed, sunflower seed, and flaxseed diets were 6.50, 13.50, 2.20, 5.20, 6.30, and 5.30 × 105 per ml rumen fluid repectively ($p < .001$). Sources of fat other than the crystalized fat caused a decrease in total VFA level, ($p < .05$) because of the lower ruminal acetate ($p < .05$) and butyrate concentrations ($p < .001$). Since the concentration of propionate in the rumen fluid was not significantly affected by different fat sources, the acetate:propionate ratio was also low ($p < .05$). In a beef cattle study (Panyakaew et al., 2013), the effects of animal fat, coconut oil, and krabok oil containing the same level of C12: 0 on the level of rumen VFA were studied, and both coconut and krabok oil increased the ruminal total VFA, especially the propionic acid level, while the acetic acid levels decreased ($p < .05$). Studies conducted by Jordan et al. (2006) have shown results similar to those reported above, suggesting that coconut oil and dried coconut

Table 4

Rumen Fermentation in Kivircik Lambs Fed With/Without MCFA

	Groups					Interaction Effects		
	Control (50:50 F/C ratio)	10 MCFA (50:50 F/C ratio)	18 MCFA (30:70 F/C ratio)	SED	*p*	Feed (F)	Measure (M)	FxM
Rumen pH								
Day 0	6.03^{b}	6.37^{ab}	6.53^{a}	.08	.025			
Day 28	6.10	6.30	6.33	.05	.073	.012	.730	.565
Day 56	6.19	6.20	6.40	.06	.336			
Intra-treatment sig	.649	.761	.272					
Rumen ammonia-N, mg/dL								
Day 0	13.73	12.38^{y}	12.72	.28	.114			
Day 28	14.33	13.10^{x}	13.58	.32	.310	.018	.018	.152
Day 56	15.48^{a}	13.78^{bx}	12.69^{b}	.39	.007			
Intra-treatment sig	.120	.031	.221					
VFA concentrations, mol/100 mol of total VFA								
Acetate								
Day 0	78.3	75.5^{x}	76.6^{x}	2.21	.883			
Day 28	78.4^{a}	64.2^{by}	62.8^{by}	3.00	.050	.035	.000	.297
Day 56	68.6	58.6^{z}	55.4^{z}	2.93	.136			
Intra-treatment sig	.363	.021	.001					
Propionate								
Day 0	25.05	24.99	24.09^{x}	.89	.894			
Day 28	26.48^{a}	28.04^{a}	20.67^{by}	.98	.002	.050	.012	.087
Day 56	25.31^{a}	21.49^{a}	17.83^{bz}	1.12	.043			
Intra-treatment sig	.740	.113	.005					
Butyrate								
Day 0	8.94^{y}	9.73^{y}	9.45	.31	.591			
Day 28	16.56^{ax}	12.09^{bx}	12.02^{b}	1.23	.014	.011	.000	.000
Day 56	20.53^{ax}	13.72^{bx}	11.24^{b}	1.64	.032			
Intra-treatment sig	.033	.019	.062					
Total VFA								
Day 0	116.3	114.1	111.7	2.40	.747			
Day 28	117.4	107.6	111.0	1.91	.642	.089	.029	.270
Day 56	117.1^{a}	97.5^{ab}	88.3^{b}	3.02	.027			
Intra-treatment sig	.983	.153	.084					

Note: $^{a, b, c}$Means in the same line with different superscripts are significantly different.
$^{x, y, z}$Means in the same line column different superscripts are significantly different.
F/C = forage:concentrate; VFA = volatile fatty acid.

oil, used as methane inhibitors, cause a decrease in the molar concentration of total rumen VFA, which is likely to be associated with ruminal H^{+} decline (Dohme et al., 1999). There were studies that showed a decrease in the level of propionic acid in animals fed with coconut oil (Bozzolo et al., 1993), and researchers have reported that this may be associated with better salivation and longer fermentation times with increased hay intake with age.

The mean values and significance controls of the carcass traits in the control and experimental groups are given in Table 2. There was no significant difference between the groups in terms of

live weights before slaughter ($p > .05$), carcass pH_0 and pH_{24}, hot and cold carcass weights, and carcass yields ($p > .05$). Consumer-accepted, quality meats have a pH value of between 5.50 and 5.80. As the carcass pH increases above those values, the risk of deterioration of the meat increases (McGeehin et al., 2001). In the present study, the hot carcass yield calculated according to pre-slaughter weight was found at 46.1–47.98%. Similarly, Bozzolo et al. (1993) found that the carcass weights of lambs fed with 5% coconut oil (51.80 kg and 52.00 kg) were not different from the control group ($p > .05$). Similar results reported by Dutta et al. (2008) on Muzafarnagari lambs fed concentrate feed supplemented with palm oil, ranging from 25 to 100 g/kg, resulted in similar carcass characteristics. Similarly, Manso et al. (2006) also observed similar carcass weights after feeding with palm oil. There was no difference between pre-slaughter weights, hot carcass weights, and carcass yields of lambs fed with rations supplemented with coconut oil at different levels (0, 25,50, 75 g/kg) ($p > .05$). The carcass yield of sheep fed with flaxseed and sunflower seed was found to be lower than that of the other groups (Peng et al., 2010). No statisticant difference was found between the carcass parameters such as cold carcass, hot carcass, hot carcass yield, and cold carcass yield of the lambs fed fish and soybean oil rations at different ratios (Ferreira et al., 2014). The authors concluded that the lambs having similar final body weight caused the absence of treatment effects on hot and cold carcass weight. In the current study, a forage:concentrate ratio of 50:50 was applied in the control and 10MCFA groups, and 70:30 in the 18 MCFA group. There were a few studies about lambs fed different forage:concentrate ratios supplemented with oils and fatty acid supplement. The 0% and 6% animal fat additions did not affect the area of M. longissimus dorsi (MLD) and carcass yield. The addition of fat to high forage ration increased the MLD muscle area but did not affect the carcass yield ($p > .05$; Zinn & Plascencia, 1996). Diets that contained different forage:concentrate feed ratios (.65:.35, .40:.60, and .10:.90) supplemented with different levels (0 and 350 g/day) of coconut oil showed that carcass weight was negligible ($p < .001$), but that fat addition had no effect on carcass weight (Lovett et al., 2003).

It can be concluded that adding MCFA to lamb diet has an effect on rumen metabolism, and the results showed that propionic acid content in the rumen fluid increased when used at the rate of .1% in 50:50 forage: concentrate diets, but cholesterol content decreased when added to rations at the rate of .18% in diets of 70:30 forage:concentrate ratios. Further studies with different inclusion rates are needed to confirm the effects of MCFA in lambs.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University (Date: February 25, 2020, No: 3).

Peer Review: Externally peer-reviewed.

Author Contributions: Concept – D.A.; Design – D.A.; Supervision – G.D.; Materials – D.A. Analysis and/or Interpretation – D.A., G.D.; Literature Search – D.A.; Writing Manuscript – D.A.

Acknowledgment: The authors are grateful to Prof. Dr. Hakan Biricik for VFA analysis and Prof. Dr. Bülent Ekiz for the statistical analysis.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This study is summarized from the doctoral thesis (supported by İstanbul University-Cerrahpaşa Research Fund with the project number 11523).

References

Alderman, G. (1985). Prediction of the energy value of com pound feeds. In W. Haresing & D. J. A. Cole (Eds.), *Recent advances in animal nutrition* (pp. 3–52). London: Butterworths.

AOAC (2005). *Official methods of analysis* (18th ed.). Association of Official Analytical Chemists

Bayat, A. R., Ventto, L., Kairenius, P., Stefański, T., Leskinen, H., Tapio, I., & Shingfield, K. J. (2017). Dietary forage to concentrate ratio and sunflower oil supplement alter rumen fermentation, ruminal methane emissions, and nutrient utilization in lactating cows. *Translational Animal Science*, *1*, 277–286. [CrossRef]

Bhatt, R. S., Soren, N. M., Tripathi, M. K., & Karim, S. A. (2011). Effects of different levels of coconut oil supplementation on performance, digestibility, rumen fermentation and carcass traits of Malpura lambs. *Animal Feed Science and Technology*, *164*, 29–37. [CrossRef]

Bozzolo, G., Bouillier-Oudot, M., Candau, M., El Hasbani, G., Grasset, D., & Manse, H. (1993). Effect of coconut oil in the post-weaning starter diet on growth and carcass qualities of maile lambs, weaned early and intensively fattened in winter. *Reproduction Nutrition Development*, *33*, 165–181. [CrossRef]

Chaney, A. L., & Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clinical Chemistry*, *8*, 130–132. [CrossRef]

Cripps, A. W., Husband, A. J., Scicchitano, R., & Sheldrake, R. F. (1985). Quantification of sheep IgG1, IgG2, IgA, IgM and albumin by radioimmunoassay. *Veterinary Immunology and Immunopathology*, *8*, 137 – 147. [CrossRef]

Demeyer, D. I. (1981). Rumen microbes and digestion of plant cell walls. *Agriculture and Environment*, *6*, 295–337. [CrossRef]

Demirel, G., Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Wood, J. D., & Enser, M. (2004). Effects of dietary n 3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. *British Journal of Nutrition*, *91*, 551–565. [CrossRef]

Dohme, F., Machmuller, A., Estermann, B. L., Pfister, P., Wasserfallen, A., & Kreuzer, M. (1999). The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. *Letters in Applied Microbiology*, *29*, 187–192. [CrossRef]

Doreau, M., & Chilliard, Y. (1997). Digestion and metabolism of dietary fats in farm animals. *British Journal of Nutrition*, *78*, S15–S35. [CrossRef]

Dutta, T. K., Agnihotri, M. K., & Rao, S. B. N. (2008). Effect of supplemental palm oil on nutrient utilization, feeding economics and carcass characteristics in post weaned Muzafarnagari lambs under feedlot conditions. *Small Ruminant Research*, *78*, 66–73. [CrossRef]

Ferreira, E. M., Pires, A. V., Susin, I., Gentil, R. S., Parente, M. O. M., Nolli, C. P., & Ribeiro, C. V. D. M. (2014). Growth, feed intake, carcass characteristics, and meat fatty acid profile of lambs fed soybean oil partially replaced by fish oil blend. *Animal Feed Science and Technology*, *187*, 9–18. [CrossRef]

Firkins, J. L., & Eastridge, M. L. (1994). Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production. *Journal of Dairy Science*, *77*, 2357–2366. [CrossRef]

Ghafari, H., Rezaeian, M., Sharifi, S. D., Khadem, A. A., & Afzalzadeh, A. (2016). Effects of dietary sesame oil on growth performance and fatty acid composition of muscle and tail fat in fattening Chaal lambs. *Animal Feed Science and Technology*, *220*, 216–225. [CrossRef]

Goering, H. K., & Van Soest, P. J. (1970). Forage fiber analysis. *USDAARS agriculture Handbook No. 379*. Government Printing Office.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., & Hervás, G. (2008). Milk production, conjugated linoleic acid content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. *Journal of Dairy Science*, *91*, 1560–1569. [CrossRef]

Hermans, D., Martel, A., Garmyn, A., Verlinden, M., Heyndrickx, M., Gantois, I., & Pasmans, F. (2012). Application of medium-chain fatty acids in drinking water increases Campylobacter jejuni colonization threshold in broiler chicks. *Poultry Science*, *91*, 1733–1738. [CrossRef]

Hong, S. M., Hwang, J. H., & Kim, I. H. (2012). Effect of medium-chain triglyceride (MCT) on growth performance, nutrient digestibility, blood characteristics in weanling pigs. *Asian-Australasian Journal of Animal Sciences*, *25*, 1003–1008. [CrossRef]

Hristov, A. N., Vander Pol, M., Agle, M., Zaman, S., Schneider, C., Ndegwa, P., Vaddella, V. K., Johnson, K., Shingfield, K. J., & Karnati, S. K. (2009). Effect of lauric acid and coconut oil on ruminal fermentation, digestion, ammonia losses from manure, and milk fatty acid composition in lactating cows. *Journal of Dairy Science*, *92*, 5561–5582. [CrossRef]

Jambrenghi, A. C., Colonna, M. A., Giannico, F., Cappiello, G., & Vonghia, G. (2007). Effect of goat production systems on meat quality and conjugated linoleic acid (CLA) content in sucking kids. *Italian Journal of Animal Science*, *6*, 612–614. [CrossRef]

Jordan, E., Lovett, D. K., Monahan, F. J., Callan, J., Flynn, B., & O'Mara, F. P. (2006). Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. *Journal of Animal Science*, *84*, 162–170. [CrossRef]

Jouany, J. P., Demeyer, D. I., & Grain, J. (1988). Effect of defaunating the rumen. *Animal Feed Science and Technology*, *21*, 229–265. [CrossRef]

Kongmun, P., Wanapat, M., Pakdee, P., Navanukraw, C., & Yu, Z. (2011). Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. *Livestock Science*, *135*, 84–92. [CrossRef]

Lovett, D., Lovell, S., Stack, L., Callan, J., Finlay, M., Conolly, J., & O'Mara, F. P. (2003). Effect of forage/concentrate ratio and dietary coconut oil level on methane output and performance of finishing beef heifers. *Livestock Production Science*, *84*, 135–146. [CrossRef]

Machmüller, A. (2006). Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. *Agriculture, Ecosystems and Environment*, *112*, 107–114. [CrossRef]

Machmüller, A., & Kreuzer, M. (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Canadian Journal of Animal Science*, *79*, 65–72. [CrossRef]

Machmüller, A., Ossowski, D. A., & Kreuzer, M. (2000). Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. *Animal Feed Science and Technology*, *85*, 41–60. [CrossRef]

Manso, T., Castro, T., Mantecón, A. R., & Jimeno, V. (2006). Effects of palm oil and calcium soaps of palm oil fatty acids in fattening diets on digestibility, performance and chemical body composition of lambs. *Animal Feed Science and Technology*, *127*, 175–186. [CrossRef]

McGeehin, B., Sheridan, J. J., & Butler, F. (2001). Factors affecting the pH decline in lamb after slaughter. *Meat Science*, *58*, 79–84. [CrossRef]

Muci, M. R., Cappello, A. R., Vonghia, G., Bellitti, E., Zezza, L., & Gnoni, G. V. (1992). Change in cholesterol levels and in lipid fatty acid composition in safflower oil fed lambs. *International Journal for Vitamin and Nutrition Research. Internationale Zeitschrift Fur Vitamin- und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition*, *62*, 330–333.

NRC (National Research Council) (2001). *Nutrient requirements of dairy cattle* (7th rev. ed), Subcommittee on dairy cattle nutrition, committee on animal nutrition, board on agriculture and natural resources. National Research Council, National Academy Press.

Ozdamar, K. (2013). SPSS ile Biyoistatistik. *10 ed. Eskişehir: Nisan Kitabevi*.

Panyakaew, P., Goel, G., Lourenço, M., Yuangklang, C., & Fievez, V. (2013). Mediumchain fatty acids from coconut or krabOK oil inhibit in vitro rumen methanogenesis and conversion of non-conjugated dienoic biohydrogenation intermediates. *Animal Feed Science and Technology*, *180*, 18–25. [CrossRef]

Peng, Y. S., Brown, M. A., Wu, J. P., & Liu, Z. (2010). Different oilseed supplements alter fatty acid composition of different adipose tissues of adult ewes. *Meat Science*, *85*, 542– 549. [CrossRef]

Ponnampalam, E. N., Mann, N. J., & Sinclair, A. J. (2006). Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts, potential impact on human health. *Asia Pacific Journal of Clinical Nutrition*, *15*, 21–29.

Senel, H. S. (1993). *Hayvan besleme*. Istanbul: Gur-Ay Matbaası.

Sondakh, E. H. B., Yusiati, L. M., Hartadi, H., & Suryanto, E. (2012). Bungkil kelapa sumber medium chain fatty acids dalam pakan ruminansia sebagai agensia penurun gas metan pada fermentasi rumen secara in vitro. *Agrinimal*, *2*, 39–43.

Sundstol, F. (1993). Energy systems for ruminants. Buvisindi. *Icelandic Agricultural Science*, *7*, 11–19.

Sutton, J. D., Knight, R., McAllan, A. B., & Smith, R. H. (1983). Digestion and synthesis in the Rumen of sheep given diets supplemented

with free and protected oils. *British Journal of Nutrition*, *49*, 419–432. [CrossRef].

Swenson, M. J. (1977). Blood circulation and the cardiovascular system. In M. J. Swenson (Ed.), *Dukes' Physiology of Domestic Animals* (9th ed.). Comstock Publishing Association, Cornell University Press.

Toprak, N. N. (2015). Do fats reduce methane emission by ruminants? - A review. *Animal Science Papers and Reports*, *33*, 305–321.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Method for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597. [CrossRef]

Van Zyl, L. C. (1967). Contributions to the study of blood constituents in domestic animals in South Africa. 6. Normal values for serum protein fractions in sheep as obtained by electrophoresis on cellulose acetate strips. *Onderstepoort Journal of Veterinary Research*, *34*, 633– 646.

Yeom, K. H., Schonewille, J. T., Everts, H., Zoet, J. M., & Beynen, A. C. (2003). Impact of dietary soybean oil versus medium-chain triglycerides on plasma fatty acids in goats. *Small Ruminant Research*, *48*, 201–208. [CrossRef]

Zinn, R. A., & Plascencia, A. (1996). Effects of forage level on the comparative feeding value of supplemental fat in growing-finishing diets for feedlot cattle. *Journal of Animal Science*, *74*, 1194–1201. [CrossRef]