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Influence of Copra Meal in the Lambs Diet on In Vitro Ruminal Kinetics and Greenhouse Gases Production

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Abstract: The present study aimed to evaluate the effect of copra meal (the waste coconut of the oil industry) on in vitro ruminal kinetic and greenhouse gases production and on in vivo lamb performance. Twenty-eight male Rambouillet sheep (initial body weight 24.5 ± 3.9 kg) were randomly assigned to one of the four treatments: 0, 50, 100, and 150 g of copra meal/kg in their diet (dry matter basis). Final weight, weight gain, and feed intake were not affected ($p > 0.05$) by the copra meal addition. The gas production volume (V) decreased, and the gas production rate increased, in a linear trend ($p < 0.05$) as copra meal was added to the diet. In contrast, methane and CO_2 production showed an opposite quadratic trend ($p < 0.05$), with the highest and lowest values reported at 100 g/kg DM of copra meal, respectively. The addition of copra meal in the lambs' diet decreases the volume of gas production and is a strategy to decrease methane and carbon dioxide production in feeding without affecting animal performance.

Keywords: coconut; methane; carbon dioxide; ruminant production

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1. Introduction

Sheep, like other ruminants, use fibrous materials and convert them into high nutritional value products (meat and milk) [1]. However, in tropical countries, due to climatic seasonality conditions, the availability and quality of forage resources are really variable [2]. Therefore, animal feed is based on agro-industrial waste available in different regions of the world, which constitutes a viable alternative from a productive and economic perspective [3]. Copra meal is one of the alternative by-products produced mainly in some tropical areas of the world [4]. Copra meal is obtained from the solid part of the endosperm of the nut and is reduced to pieces. It is usually considered waste, and it is mentioned that large quantities of this are left to rot in the fields as unwanted material [5]. Some reports have calculated an energy value equivalent to 2.5 kg of corn and sorghum, which makes it attractive for ruminants feed due to its energy value, 4.7 Mcal/kg DM (Dry Matter). In this sense, González-Garduño et al. [6] reported that supplementation with coconut paste increases weight gains in lambs at grazing; these results may be due to high oil content.

Methane (CH_4) and carbon dioxide (CO_2) are potent greenhouse gases [7]. These gases contribute 14.3% and 73.9%, respectively, to anthropogenic global greenhouse gases [7]. In addition, 17% to 37% of global anthropogenic methane (CH_4) emissions have been estimated to result from ruminant livestock [8], and practices related to livestock production are estimated to produce around 10.8% of the global greenhouse emissions [9,10].

Other research has reported that the use of plant oils reduces enteric CH_4 emissions via ruminal defaunation [11], toxic effects on methanogens [12], and decreased diet digestibility [13]. Jordan et al. [12] identified that 250 g of refined coconut oil/d fed to beef heifers (approximately 500 kg of BW) could reduce enteric CH_4 emissions and positively affect the fatty acid profile in the muscle produced in terms of human nutrition health. High quantities of coconut oil can be retained in copra meal if the industrial oil extraction process is inefficient [12]. Jordan et al. [13] observed that coconut oil in copra meal might influence ruminal fermentation and promote a reduction in methane production, as was observed when refined coconut oil was used in diets [11,13]. Thus, the current study aimed to evaluate the effect of copra meal on in vitro ruminal kinetic and carbon dioxide and methane gas production.

2. Materials and Methods

2.1. Productive Phase

The experimental trial was conducted at the lambs experimental and production unit of UASLP, Mexico. Four experimental diets (Table 1) of 0, 50, 100, and 150 g of copra meal kg^{-1} DM were randomly assigned to 28 Rambouillet lambs (initial body weight 24.5 ± 3.9 kg) housed in individual cages in a naturally ventilated barn. Balanced diets were according to the nutritional requirements by NRC [14]. The diets were provided at 08:00 and 15:00 h each day. At the beginning of the experiment, lambs were adapted to the diets for seven days; experimental investigation began after this period and lasted for 42. Feed intake and orts were measured daily. Every 14 days, lambs were weighed before their morning feeding. The feed conversion was calculated as the ratio of daily intake to daily weight gain.

Table 1. Ingredients and chemical composition of the experimental diets.

Ingredients, g kg^{-1} of DM	**Copra Meal g kg^{-1} of DM**			
	0	**50**	**100**	**150**
Alfalfa Hay	285	275	230	180
Corn Stover	150	160	160	160
Corn Grain	270	250	255	255
Ground Sorghum	150	130	130	130
Soybean Meal	50	40	30	30
Copra Meal	0	50	100	150
Cane Molasses	75	75	75	75
Urea	15	15	15	15
Minerals Premix [1]	5	5	5	5
Chemical composition, g kg^{-1} of DM				
Dry Matter	898	899	898	890
Crude Protein	164	166	166	167
Ash	154	176	139	137
Ether Extract	330	343	371	375
NDF	346	301	329	314

[1] 1 kg of mineral mix contains: Ca 240 g, P 30 g, Mg 20 g, Na 80 g, Cl 120 g, K 5 g, S 5 g, lasalocid 2000 mg, Mn 4000 mg, Fe 2000 mg, Zn 5000 mg, Se 30 mg, Co 60 mg, vitamin A 500,000 IU, vitamin D 300,000 IU, and vitamin E 1000 IU.

Samples of the experimental diets were taken every 14 days. The experimental diets were analyzed for their chemical composition and dry matter (DM), crude protein (PC) according to the AOAC [15], neutral detergent fiber (NDF), and acid detergent fiber (ADF) [16].

2.2. In Vitro Gas Production

Experimental diets were used as substrates for in vitro incubations formulated in an isoproteic and iso-energetic manner. On the other hand, a sample of 100 g from each diet was taken for its later bromatological analysis. The samples were dried at 65 °C for

24 h. Once dried, the samples were milled to a sieve with a mesh 1 mm in diameter. For incubation, 500 mg of each diet sample was incorporated into 90 mL of culture medium.

The reduced (without oxygen) culture media per liter used in vitro contained (Cobos and Yokoyama [17]): mineral solution A, 6 g of potassium hydrogen phosphate was added to 1 L of distilled water; mineral solution B, 6 g of potassium hydrogen phosphate, 1.6 g calcium chloride, 6 g ammonium sulfate, 12 g sodium chloride 2.45 g magnesium sulfate, and 18% sodium carbonate buffer solution, were added to 1 L of distilled water; and a cysteine reduced solution, where 2.5 g of sodium sulfide and 0.1 mL of resazurin (1%) was mixed with mineral solutions and CO_2 pumped until the marker turned.

Two Rambouillet rams, fed a diet of 50% corn grain plus 50% corn silage, were ruminal fluid donors. The ruminal fluid (10 mL) and the culture media (90 mL) were added to the amber flask [18] that contained 500 mg of the diet. These samples were incubated at 39 °C in a water bath. Ten repetitions of each diet and three blanks for correction were used.

2.3. Gas Production

The pressure was measured at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, and 48 h to calculate the volume and accumulated gas production, as based on a logistic model [18]:

$$Vo = v/1 + \exp(2\text{-}4 * s * (t - L)) \quad (1)$$

where, Vo = total gas produced, v = volume, s = gas production rate, t = time, and L = lag phase.

2.4. In Vitro Dry Matter Degradability (IVDMD)

At the end of incubation (96 h), each Ankom bag (n = 5) was washed and then dried for 48 h at 65 °C to calculate the level of DM disappearance.

Using the model developed by Menke and Steingass [18], the metabolizable energy (ME, MJ/kg DM) was estimated as:

$$ME = 2.20 + 0.1357GP24 + 0.0057CP + 0.0002859EE2 \quad (2)$$

where, GP24 was the amount of gas produced after 24 h of incubation, CP was the crude protein of diets (% DM) and EE was the Ether Extract of diets (%DM).

The profiles of short-chain fatty acids (SCFA) were calculated according to the equation proposed by Getachew et al. [19]:

$$mmolSCFA = -0.00425 + 0.0222 \text{ (mL gas at 24 h)} \quad (3)$$

2.5. Carbon Dioxide (CO_2) and Methane (CH_4) Estimation

Sequential selection of each tube's outflow to an infrared gas analyzer (IRGA) attached to a custom chamber (Li-6400XT and 6400-09, LiCor, Lincoln, NE, USA) allowed for the monitoring of CO_2 mixing ratios in real-time. Later, the production levels of CO_2, at 4, 8, 12, 24, 36, 48, and 72 h of fermentation, were measured. Estimations of the methane levels were developed through the analysis of the proportions in these measured CO_2 values [20] and the time that this proportion was calculated [21].

2.6. Statistical Analysis

The data obtained were analyzed using a completely randomized design [22]. Orthogonal polynomial contrasts were used to verify linear or quadratic effects for the copra meal levels. The PROC NLIN BEST method (SAS Institute, 2002, NC, USA) was performed to calculate the ruminal in vitro kinetics (volume of gas produced, gas production rate, and initial gas production). Data were analyzed with JMP7 software [23]. A *p*-value of 0.05 was selected as the significance level.

3. Results

3.1. Productive Test

The effect of copra meal on final weight, dry matter intake, average daily gain, and feed conversion was not statistically significant. The lambs showed similar growth performance with fed diets of 0, 50, 100, and 150 g (Table 2). Final weight, weight gain, and feed intake were lower in lambs whose diets 100 g/kg MS of copra meal than in other treatment conditions.

Table 2. Performance of lambs fed with diets that contained different levels of copra meal.

Item	Copra Meal g kg^{-1} DM					*p*-Value	
	0	50	100	150	SEM ‡	l ¥	q ¥
Initial weight, kg^{-1}	26.48	30.16	28.08	26.72	1.34	0.92	0.23
Final weight, kg^{-1}	37.84	41.40	35.80	38.20	2.41	1.23	1.85
Dry matter intake, kg^{-1}	1.32	1.37	1.24	1.33	0.05	0.67	0.34
Daily live weight gain g/day	0.25	0.24	0.21	0.25	0.31	0.73	0.50
Feed conversion	5.48	5.71	6.90	5.67	0.80	0.54	0.82

‡ SEM, standard error of the mean; l ¥, linear effect; q ¥, quadratic effect.

3.2. In Vitro Dry Matter Degradability (IVDMD)

A quadratic trend ($p < 0.05$) was found for the in vitro digestibility of dry matter (IVDMD) between treatments at 0 h and 48 h; however, at 96 h IVDMD, a tendency was not detected (Figure 1).

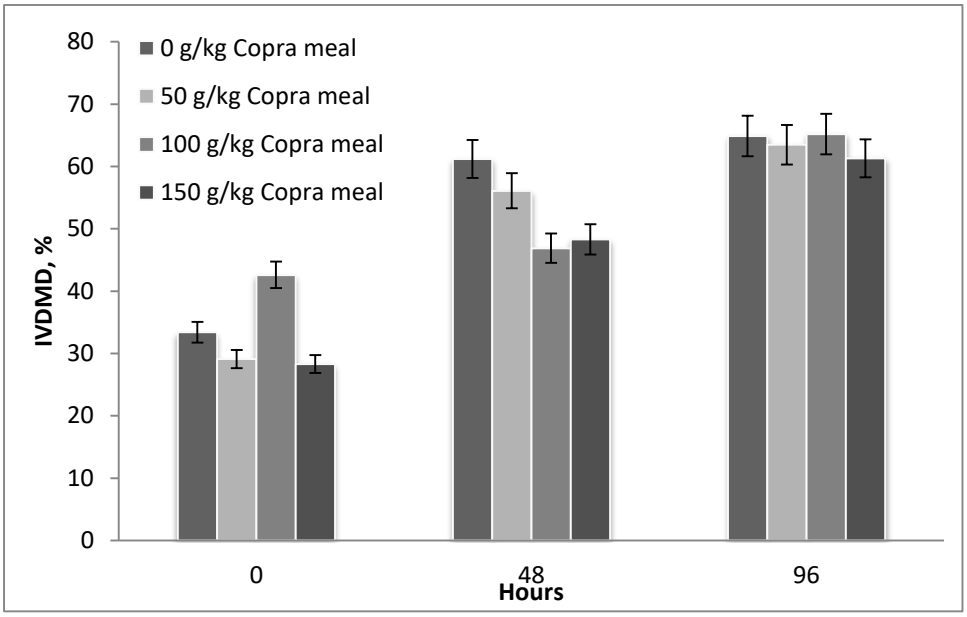

Figure 1. In vitro dry matter digestibility of diets with different amounts of copra meal at 0, 48, and 96 h. The *p*-values of the linear and quadratic effects: at 0 h (0.51 and 0.04), 48 h (0.03 and 0.9), and 96 h (>0.05 and >0.05).

3.3. In Vitro Gas Production

Gas production volume (Vm) was affected in a linear way by the addition of copra meal ($p < 0.05$). Moreover, the gas production rate (s) increased linearly ($p < 0.05$) as the amount of copra meal increased. The lag phase (L) showed no difference ($p < 0.05$) between treatments (Table 3). The production of methane presented a quadratic effect ($p < 0.05$), having a maximum production value (34.03%) in the diet with 100 g/kg MS of copra meal. In the case of carbon dioxide, a quadratic effect ($p < 0.05$) was also registered, but with a minimum value (65.96%) in the diet with 100 g/kg MS of copra meal. Although no

differences ($p < 0.05$) were observed for the values of short-chain fatty acids (SCFA) or metabolizable energy (ME), a numerical increase in metabolizable energy was observed for the diet with 100 g/kg MS of copra meal (Table 3).

Table 3. In vitro gas production kinetics for feed with different levels of copra meal.

Item	Copra Meal g kg^{-1} DM					*p*-Value	
	0	50	100	150	SEM ‡	l ¥	q ¥
V, mL g^{-1}	173.8	179.05	188.7	153.02	4.51	0.03	0.82
s, mL g^{-1}	0.029	0.029	0.031	0.3	0.01	0.02	0.44
L, h	5.27	4.94	5.17	5.68	0.25	0.61	0.87
Dioxide Carbon (CO_2), %	68.79	66.84	65.96	72.98	1.78	0.17	0.04
Methane (CH_4), %	31.2	33.15	34.03	27.01	1.29	0.94	0.01
ME, MJ kg^{-1} MS	15.49	15.99	17.32	14.20	1.30	0.94	0.23
SCFA mmol L^{-1}	2.16	2.24	2.46	1.95	0.01	0.58	0.66

V, maximum volume; L, lag phase; s, gas production rate; ME, metabolizable energy; SCFA, short-chain fatty acids; ‡ SEM, standard error of the mean; l ¥, linear effect; q ¥, quadratic effect.

4. Discussion

4.1. Productive Test

Copra meal had no effect on the final weight, dry matter intake, average daily gain, or feed conversion of the lambs (Table 2). This effect could be comparable with the results of beef heifers supplemented with copra meal by Jordan et al. [12]. Jordan et al. [12,13] did not observe changes in food consumption when maintaining the diet at isotropic and iso-energetic levels. Jordan et al. [13] concluded that the amounts of other ingredients (alfalfa, sorghum, soybean meal) are modified, and the animals could reach a steady state. Although a difference in production parameters was not observed, the inclusion of 100 g/kg MS of copra meal in diets showed the lowest weight gains. In contrast with our results, Jordan et al. [12] reported an improvement for these variables. We theorized that the improvements could be attributed to maintaining the same level of consumption, as the oil content would increase the animal's energy content and decrease energy loss in the form of methane [24].

4.2. In Vitro Dry Matter Degradability (IVDMD) and In Vitro Gas Production

A quadratic effect was observed for IVDMD during 0 h to 48 h. However, at 96 h, no changes were detected (Figure 1). Lima et al. [25] observed that the in vitro digestibility of organic matter ranged from 35.91 to 61.18% in diets of 0 to 30% copra meal. The maxi-mum degradability was found among the control group, coinciding with the values found in this research. The fat content in copra meal affects digestibility because it forms a barrier between the ruminal microorganisms and feed particles [26].

Copra meal increases linearly with the gas production volume and rate (Table 3). The diet that contained 100 g/kg MS showed a maximum gas production of 188.7 mL g^{-1}, a value higher than in any other treatment condition. However the treatment with 150 g kg^{-1} MS showed the minimum gas production level of 153.2 mL g^{-1}, thus confirming the potential of coconut oil to inhibit ruminal fermentation, as has been previously recognized [25]. Hollmann and Beede [27] mention that diets with 2.5% coconut oil decreased milk production, correlating with decreased dry matter digestibility and neutral detergent fiber.

The production of methane showed a quadratic reduction in its gas production value in the diet with 100 g/kg MS of copra meal. Similarly, the copra meal reduced the carbon dioxide gas production value when diets with 100 g/kg MS were used. A reduction in methane production as copra meal increased could be explained through the high-fat content diets that reduce methanogenesis by inhibiting the activity of protozoa and archaeans. These diets also inhibit the biohydrogenation of unsaturated fatty acids that act as hydrogen receptors [28,29]. Soliva et al. [30] demonstrated that garlic oil is more effective in mitigating methane production without decreasing nutrient utilization efficiency. O'

Brien et al. [31] indicated that the use of saturated fatty acids could be an efficient strategy to mitigate methane production from inhibition in in vitro ruminal fermentation, considering the diet–dose correlation. It could also be considered to mitigate carbon dioxide production by enteric digestion, as reveal the results of this research.

5. Conclusions

The addition of copra meal to a diet, under in vitro conditions, decreases the gas production volume equal to that of methane and carbon dioxide production. However, it does not affect the lamb's performance.

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References

1. Aguayo-Ulloa, L.; Lama, G.; Pascual-Alonso, M.; Olleta, J.; Villarroel, M.; Sañudo, C.; María, G. Effect of enriched housing on welfare production performance and meat quality in finishing lambs: The use of feeder ramps. *Meat Sci.* **2014**, *97*, 42–48. [CrossRef] [PubMed]
2. Ku-Vera, J.; Ayala-Burgos, A.; Solorio-Sánchez, F.; Briceño-Poot, E.; Ruiz-González, A.; Piñeiro-Vázquez, A.; Barros-Rodríguez, M.; Soto-Aguilar, A.; Espinoza-Hernández, J.; Albores-Moreno, S.; et al. Tropical tree foliages and shrubs as feed additives in ruminant rations. In *Nutritional Strategies of Animal Feed Additives*; Nova Sci. Publishers: New York, NY, USA, 2013; pp. 59–76.
3. Rodrigues, L.S.; Menezes, B.P.; Silva, A.G.M.; Faturi, C.; Silva, J.A.R.; Garcia, A.R.; Nahúm, B.S.; Andrade, S.J.T.; Lourenço Junior, J.B. Ovine feed intake digestibility and nitrogen balance in feeds containing different amounts of cupuaçu meal. *Semin. Cienc. Agrar.* **2015**, *36*, 2799–2808. [CrossRef]
4. Stein, H.H.; Lagos, L.V.; Casas, G.A. Nutritional value of feed ingredients of plant origin fed to pigs. *Anim. Feed Sci. Tech.* **2016**, *218*, 33–69. [CrossRef]
5. Ng, S.P.; Tan, C.P.; Lai, O.M.; Long, K.; Mirhosseini, H. Extraction and characterization of dietary fiber from coconut residue. *J. Food Agric. Environ.* **2010**, *8*, 172–177.
6. González-Garduño, R.; Torres-Hernández, G.; Arece-García, J. Ganancia de peso de ovinos alimentados con pasto Taiwán (Pennisetum purpureum) suplementados con diversas fuentes de proteína. *AIA* **2011**, *15*, 3–20.

7. IPCC. IPCC summary for policymakers. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2014; pp. 1–32.
8. Hünerberg, M.; McGinn, S.M.; Beauchemin, K.A.; Okine, E.K.; Harstad, O.M.; McAllister, T.A. Effect of dried distillers' grains with solubles on enteric methane emissions and nitrogen excretion from finishing beef cattle. *Can. J. Anim. Sci.* **2013**, *93*, 373–385. [CrossRef]
9. O'Mara, F.P. The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future. *Anim. Feed Sci. Technol.* **2011**, *166*, 7–15. [CrossRef]
10. O'Mara, F.P. The role of grasslands in food security and climate change. *Ann. Bot.* **2012**, *110*, 1263–1270. [CrossRef]
11. Shi, L.; Zhang, Y.; Wu, L.; Xun, W.; Liu, Q.; Cao, T.; Hou, G.; Zhou, H. Moderate Coconut Oil Supplement Ameliorates Growth Performance and Ruminal Fermentation in Hainan Black Goat Kids. *Front. Vet. Sci.* **2020**, *23*, 622259. [CrossRef]
12. Jordan, E.; Lovett, D.K.; Hawkins, M.; Callan, J.J.; O'Mara, F.P. The effect of varying levels of coconut oil on intake, digestibility and methane output from continental cross beef heifers. *Anim. Sci.* **2006**, *82*, 859–865. [CrossRef]
13. Jordan, E.; Lovett, D.K.; Monahan, F.J.; Callan, J.; Flynn, B.; O'Mara, F.P. Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. *J. Anim. Sci.* **2006**, *84*, 162–170. [CrossRef]
14. NRC. *Nutrient Requirements of Small Ruminants*; The National Academies Press: Washington, DC, USA, 2007; p. 384.
15. AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 1990.
16. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
17. Cobos, P.M.; Yokoyama, M.T. *Clostridium Paraputrificum* var. *Ruminantium*: Colonization and degradation of shrimp carapaces in vitro observed by scanning electron microscopy. In *Rumen Ecology Research Planning*, 1st ed.; Wallace, R.J., Lahlou-Kassi, A., Eds.; ILRI: Addis Ababa, Ethiopia, 1995; Volume 1, pp. 151–162.
18. Menke, K.H.; Steingass, H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 9–52.
19. Getachew, G.; Makkar, H.P.S.; Becker, K. Tropical browses: Contents of phenolics compounds, in vitro gas production and stoichiometric relationship between short chain fatty acid an in vitro gas production. *J. Agric. Sci.* **2002**, *139*, 341–352. [CrossRef]
20. Singh, G.P.; Mohini, M. Effect of different levels of Rumensin in diet on rumen fermentation, nutrient digestibility, and methane production in cattle. *Asian Aust J. Anim. Sci.* **1999**, *12*, 1215–1221. [CrossRef]
21. Ferrer, I.; Garfí, M.; Uggetti, E.; Ferrer-Martí, L.; Calderon, A.; Velo, E. Biogas production in low-cost household digesters at the Peruvian Andes. *Biomass Bioenergy* **2011**, *35*, 1668–1674. [CrossRef]
22. Steel, R.G.D.; Torrie, J.H.; Dickey, D.A. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd ed.; MacGraw Hill: New York, NY, USA, 1997.
23. Sall, J.; Lehman, A.; Stephens, M.; Creighton, L. *JMP® Start Statistics: A Guide to Statistics and Data Analysis*, 5th ed.; SAS Institute Inc: Cary, NC, USA, 2012.
24. Bharanidharan, R.; Arokiyaraj, S.; Baik, M.; Ibidhi, R.; Lee, S.J.; Lee, Y.; Nam, I.S.; Kim, K.H. In Vitro Screening of East Asian Plant Extracts for Potential Use in Reducing Ruminal Methane Production. *Animals* **2021**, *11*, 1020. [CrossRef] [PubMed]
25. Lima, T.J.; Paula, T.A.; Castagnino, P.S.; Teixeira, I.A.M.A.; Beelen, R.N.; Guimarães Beelen, P.M. Kinetic and in vitro ruminal fermentation characteristics of copra meal diets with different fat levels. *Acta Vet. Bras.* **2017**, *11*, 35–41.
26. Bayat, A.R.; Tapio, I.; Vilkki, J.; Shingfield, K.J.; Leskinen, H. Plant oil supplements reduce methane emissions and improve milk fatty acid composition in dairy cows fed grass silage-based diets without affecting milk yield. *J. Dairy Sci.* **2018**, *101*, 1136–1151. [CrossRef]
27. Hollmann, M.; Beede, D.K. Comparison of effects of dietary coconut oil and animal fat blend on lactational performance of Holstein cows fed a high-starch diet. *J. Dairy Sci.* **2012**, *95*, 1484–1499. [CrossRef]
28. Malik, P.K.; Trivedi, S.; Mohapatra, A.; Kolte, A.P.; Sejian, V.; Bhatta, R.; Rahman, H. Comparison of enteric methane yield and diversity of ruminal methanogens in cattle and buffaloes fed on the same diet. *PLoS ONE* **2021**, *16*, e0256048.
29. Scarpa, G.; Tarricone, S.; Ragni, M. Carcass composition, meat quality and sensory quality of Gentile di Puglia light lambs: Effects of dietary supplementation with oregano and linseed. *Animals* **2021**, *11*, 607. [CrossRef]
30. Soliva, C.R.; Amelchanka, S.L.; Duval, S.M.; Kreuzer, M. Ruminal methane inhibition potential of various pure compounds in comparison with garlic oil as determined with a rumen simulation technique (Rusitec). *Br. J. Nutr.* **2011**, *106*, 114–122. [CrossRef]
31. O'Brien, M.; Navarro-Villa, A.; Purcell, P.J.; Boland, T.M.; O'Kiely, P. Reducing in vitro rumen methanogenesis for two contrasting diets using a series of inclusion rates of different additives. *Anim. Prod. Sci.* **2014**, *54*, 141–157. [CrossRef]