agriculture

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# Profiling of Fatty Acids and Rumen Ecosystem of Sheep Fed on a Palm Kernel Cake-Based Diet Substituted with Corn

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**Abstract:** The purpose of this study was to analyze the effects of corn substitution on the rumen functions and fatty acid profile of Dorper lambs fed a diet based on palm kernel cake (PKC). Corn was replaced with PKC basal diet at the following levels: C0% = (0% corn + 75.3% PKC), C1% = (5% corn + 70.3% PKC) and C2% = (10% corn + 65.3% PKC) of diet. The rumen fermentation was carried out in vitro, and feeding trials were in vivo. Twenty-seven lambs were used to determine gastrointestinal tract content and rumen fluid fatty acid. Rumen liquor was obtained from four fistulae Dorper sheep and incubated with 200 mg of each treatment for 24 and 72 h. In vitro organic matter digestibility (IVOMD), in vitro dry matter digestibility (IVDMD), volatile fatty acids (VFA), methane estimation ($CH_4$), rumen microbial population and fatty acid biohydrogenation were determined. The results of the in vitro study showed there were no significant differences in IVDMD, IVOMD, $NH_3$-N, pH and VFA at 72 h. Higher significant $CH_4$ production was observed in C0% when compared with C1% and C2%. Microbial population did not differ significantly between treatment groups. The rates of biohydrogenation were not affected by corn substitution, although a significant difference was observed in C18:1n9 (in vitro) and C18:1 t-11 (in vivo). In conclusion, the present study indicated that the corn substitution in the PKC diets maintained fermentation characteristics with an increase in unsaturated fatty acids in the rumen.

**Keywords:** corn; fatty acids; in vitro digestibility; palm kernel cake; rumen function



**Citation:** Saeed, O.A.; Sani, U.M.; Sazili, A.Q.; Akit, H.; Alimon, A.R.; Samsudin, A.A. Profiling of Fatty Acids and Rumen Ecosystem of Sheep Fed on a Palm Kernel Cake-Based Diet Substituted with Corn. *Agriculture* **2023**, *13*, 643. https://doi.org/10.3390/agriculture13030643

Academic Editor: Secundino López

Received: 25 October 2022
Revised: 15 February 2023
Accepted: 16 February 2023
Published: 9 March 2023



## 1. Introduction

The high cost of grains has forced farmers to seek an alternative source to feed for their livestock. South-East Asian countries such as Malaysia and Indonesia have abundant oil palm resources, resulting in a large volume of oil palm byproducts such as palm kernel cake (PKC). Palm kernel cake is now recognized as an important and cost-effective source of high-quality ruminant feed [1]. It is also a rich source of protein in the diet that could be a cost-effective feed and fattening strategy in comparison with imported concentrate feed [2]. Palm kernel cake was reported to have between 16–18% crude protein, which is considered to be moderately digestible and suitable for livestock's feeding [3]. Chanjula, et al. [4] reported an inclusion level of 35% PKC in the diets of goats on a digestibility trial that lasted 21 days with no adverse effects on rumen fermentation characteristics, nutrient utilization, or microbial populations. However, corn has been a good source of energy in ruminants' diets which can influence growth performance. Recently, corn supplementation has become a common practice in order to increase the energy density of diets for high-production ruminants [5–7]. The inclusion of corn in diets in the current study could compensate for the lack of energy that may occur when feeding on high levels of PKC. We hypothesized that the partial inclusion of corn in a PKC-based diet could

increase the rate of fermentation [8]. Furthermore, there is a lack of information regarding the utilization of PKC as a primary constituent of high-concentrate diets that also include corn for in vitro fermentation and in vivo trials. Therefore, the purpose of this investigation was to evaluate the rumen digestibility and fermentation parameters of a PKC-based diet with varying amounts of corn as a substitute.

## 2. Materials and Methods

### *2.1. Experimental Design of Study*

Two experiments were conducted, involving in vitro rumen fermentation and in vivo feeding trials. The in vivo experiment was carried out at the small ruminant field of Universiti Putra Malaysia. The in vivo experiment used a total of twenty-seven Dorper crossbred lambs, each about six months old and weighing about 15 ± 0.59 kg, which were randomly assigned to one of three dietary treatments (nine lambs per treatment). Individual pens with feeders and water bowls were provided for the lambs. The specific objective of the in vivo study was to determine gastrointestinal tract content and rumen fluid fatty acids. The in vitro study used four Dorper sheep with body weights of 28 ± 0.54 kg (mean ± standard deviation) fitted with permanent rumen fistula as rumen content donors. The aim of the in vitro trial was to study the parameters of rumen fermentation, the fatty acids in rumen fluid, and microbial population in the rumen. The animals were fed roughages and a commercial concentrate diet and kept individually in cages. The fistulae sheep were fed twice a day, water and mineral blocks were provided ad libitum. The fistulae sheep were fed a 50:50 rice straw and concentrate diet. Equal volumes of rumen liquor were obtained from the four fistulae Dorper sheep prior to morning feeding and strained through four layers of cheesecloth into a thermos flask, which was flushed with $CO_2$ and immediately transported to the laboratory for incubation. Three dietary treatments, a control diet (0% corn + 75.3% PKC), a C1% diet containing (5% corn + 70.3% PKC), and a C2% diet (10% corn + 65.3% PKC), were formulated. The chemical composition and ingredients of experimental diets are presented in Table 1. Corn starch incorporated into the diets as a source of energy was derived from corn.

**Table 1.** Ingredients and chemical compositions of the treatment diets (DM basis).

| Item | Corn Concentration (%) | | |
|---|---|---|---|
| | C0% | C1% | C2% |
| Rice straw urea treated | 20 | 20 | 20 |
| PKC | 75.3 | 70.3 | 65.3 |
| Protected fat (Megalac) | 3 | 3 | 3 |
| Corn | 0 | 5 | 10 |
| $CaCO_3$ | 1 | 1 | 1 |
| NaCl | 0.5 | 0.5 | 0.5 |
| Vitamin premix | 0.2 | 0.2 | 0.2 |
| Total | 100 | 100 | 100 |
| Chemical composition: | | | |
| DM | 91.78 | 91.66 | 91.55 |
| Ash | 13.80 | 12.72 | 12.74 |
| OM | 86.19 | 87.27 | 87.26 |
| CP | 15.42 | 14.88 | 14.09 |
| EE | 5.3 | 5.1 | 4.33 |
| CF | 26.6 | 24.50 | 20.83 |
| NDF | 62.36 | 60.06 | 55.66 |
| ADF | 45.60 | 40.96 | 37.30 |
| ADL | 6.56 | 6.10 | 5.43 |
| Gross energy (MJ/kg DM) | 16.89 | 17.29 | 17.65 |
| Calculation: | | | |
| Hemicellulose | 16.76 | 19.10 | 18.36 |
| Cellulose | 39.03 | 34.86 | 31.86 |

**Table 1.** *Cont.*

| Item | Corn Concentration (%) | | |
|---|---|---|---|
| | C0% | C1% | C2% |
| NFE | 40.44 | 41.11 | 48.39 |
| ME (MJ/kg DM) | 7.36 | 8.23 | 8.92 |
| TDN (%) | 43.91 | 49.24 | 53.46 |

The ratios of concentrate to straw were given on 80:20 dry matter basis, vitamin premix A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFE: nitrogen-free extract; ME: metabolizable energy; TDN: total digestible nutrients.

The fatty acids of experimental diets are presented in Table 2. The diets included a combination of roughage and concentrate. The lambs were fed at 8:00 a.m. and 6:00 p.m. daily with a 20:80 ratio of roughage to concentrate. After 120 days, five lambs from each treatment were slaughtered in the Meat Science Laboratory, Department of Animal Science, Agriculture Faculty, Universiti Putra Malaysia, following the standard protocol outlined in MS 1500:2004. Gentle emptying and weighing were performed on the rumen, reticulum, omasum, and abomasum after the gastrointestinal tract had been carefully dissected.

**Table 2.** Chemical and fatty acid composition of treatments.

| Fatty Acids | Diets | | |
|---|---|---|---|
| | C0% | C1% | C2% |
| C12:0 | 8.11 | 4.74 | 5.57 |
| C14:0 | 5.88 | 6.32 | 3.94 |
| C15:0 | 3.67 | 2.43 | 2.68 |
| C15:1 | 0.63 | 0.26 | 0.73 |
| C16:0 | 31.97 | 35.43 | 39.97 |
| C16:1 n-9 | 0.33 | 0.39 | 0.37 |
| C18:0 | 14.14 | 14.74 | 7.59 |
| C18:1n9 | 31.44 | 31.71 | 36.01 |
| C18:2n6 | 3.16 | 3.10 | 2.55 |
| C18:3n-3 | 1.27 | 1.09 | 1.29 |
| ΣSFA | 63.78 | 63.68 | 59.77 |
| ΣUFA | 36.21 | 36.31 | 40.22 |
| ΣMUFA | 32.41 | 32.37 | 37.12 |
| Σn-3 PUFA | 1.27 | 1.09 | 1.29 |
| Σ n-6 PUFA | 3.16 | 3.10 | 2.55 |
| Σ PUFA | 4.43 | 4.20 | 3.83 |
| n-6:n-3 Ratio | 2.71 | 2.85 | 2.18 |
| UFA:SFA | 0.56 | 0.57 | 0.67 |
| Poly:Sat Ratio | 0.07 | 0.06 | 0.07 |

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

### *2.2. Sampling and Incubations Procedures of In Vitro Experiment*

The in vitro experiment was conducted in three runs, glass syringes with internal diameters of 32 mm, lengths of 200 mm, and measured volumes of 100 mL were used for incubations. A rumen fluid-buffer medium (30 mL) was poured into a 100-mL glass syringe containing about 200 mg of dry treatment samples and fitted with a silicon tube. A blank containing only the rumen fluid-buffer mixture, not samples as well as standard hay and standard concentrate, was also incubated in syringes. Samples, blank, standard hay, and concentrate were incubated in triplicate. Two standards (hay and concentrate samples) were provided by the University of Hohenheim (Stuttgart, Germany) and were those proposed by Menke and Steingass [9]. At the end of the trials, the in vitro samples were pooled based on time and treatment (0, 24, and 72 h of incubation). Two to three drops of 10% $H_2SO_4$ were added to one tube which was used for $NH_3$-N analysis while the other tube was used for fatty acid and biohydrogenation analysis (0 and 72 h of incubation) and

microbial population (24 h of incubation). The substrate in both tubes was kept at −20 °C until analysis.

### *2.3. Rumen Fermentation Assessment*

In this study, seven samples were used for each assessment per treatment after the samples were pooled together. The in vitro dry matter digestibility (IVDMD) was ascertained at 72 h of incubation according to Menke and Steingass [9]. The in vitro organic matter digestibility (IVOMD) was determined as follows [10].

The blank and sample contained in the glass syringes were poured into a pre-weighed sintered glass, the syringes were properly rinsed with distilled water to remove all residues, and the water was evacuated completely from the sintered glass with the aid of a vacuum pump. Then, the sintered glass and its contents were dried for 24 h in an airtight oven at 105 °C. Ammonia nitrogen ($NH_3$-N) was determined in accordance with Parsons et al. [11]. A standard curve was made to determine whether a linear relationship existed between the varying concentrations of ammonium sulphate standard solution and the intensity of color produced. The intensity of color thus developed was measured at a wavelength of 420 nm using a spectrophotometer (Secomam, Domont, France) within 5–10 min after setting it at 0 absorbance with the blank. The determination of volatile fatty acid (VFA) was performed using a gas chromatograph (Hewlett Packard 6890 GC system) according to the procedure of Cottyn and Boucque [12]. The rumen fluid sample from the freezer was kept at room temperature for about 2 h to defrost and 1 mL was pipetted into a falcon tube and 200 µL of 25% (*w*/*v*) meta-phosphoric solution added and allowed to remain at room temperature for about half an hour. Then it was centrifuged at 3000 g for 10 min at 24 °C. About 500 µL of supernatant was pipetted into a pre-labeled GC vial and 500 µL of 20 Mm×4-methyl N-valeric acids were added as an internal standard. Ruminal VFA was separated and quantified by gas chromatography (Agilent 6890, Mississauga, ON, Canada). Methane generated during in vitro rumen fermentation of the feeds in the syringes was approximated utilizing the equation based on VFA proportion according to Moss, et al. [13].

$$CH_4 = 0.45 \times (A) - 0.275 \times (P) + 0.4 \times (B)$$

where,

$CH_4$ = amount (mmol) of methane produced
(A) = concentration (mmol) of acetate
(B) = concentration (mmol) of butyrate
(P) = concentration (mmol) of propionate

### *2.4. Microbial Populations and Quantification*

The microbial populations were quantified after 24 h. The extraction of the microbial populations' DNA was carried out using the QIAamp® DNA Mini Stool Kit (Qiagen, Hilden, GmbH) in accordance with the manufacturer's instructions with some little modifications. The strength of the DNA was measured using an ND-1000 NanoDrop (NanoDrop Technologies, Silverside, Wilmington, DE, USA) spectrophotometer. Species-specific quantitative real-time PCR was carried out using the CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA, USA) with an optical grade-plate for SYBR Green mix detection. The microbial populations such as total bacteria, cellulolytic bacteria, methanogenic archaea and total protozoa were determined by quantitative real-time PCR according to Saeed et al. [14]. The PCR reaction was carried out in a total volume of 25 µL using the QuantiFast SYBR® Green RT-PCR Kit. Every sample's reaction contained 12.5 µL of SYBR Green Supermix, 1 µL of Forward Primer, 1 µL of Reverse Primer, 2 µL of DNA samples, 9.5 µL of DNA template, and RNAase-free water. Axygen scientific 0.2, thin wall, transparent Real-Time PCR strips with flat clear caps were used to hold the mixed samples. Every well received the following PCR reaction conditions: a 5 min initial incubation at 94 °C,

followed by 40 cycles of denaturation at 94 °C for 20 s, annealing temperature for 30 s, and extension to 72 °C for 20 s.

### *2.5. Fatty Acid Analysis*

The rumen liquor was collected immediately after slaughtering the animal in falcon tubes for fatty acid determination. The incubated rumen substrates for 0 and 72 h were removed from the freezer and allowed to thaw at room temperature for about 45 min. The total lipid extraction method described by Folch et al. [15] was used for the extraction of fatty acids from the rumen fluid samples. The total fatty acids were derived from 10 mL rumen liquor, and fatty acids were detected using a gas chromatograph (Agilent 7890A). The rumen liquor column was made of fused silica capillaries (Supelco SP-2560, 100 m, 0.25 mm ID, 0.20 mm film thickness).

Apparent biohydrogenation of oleic acid (C18:1n-9c), linoleic acid (C18:2n-6c), and $\alpha$-linolenic acid (C18:3n-3) were determined based on the difference in the concentration of fatty acids between 0 and 72 h in vitro incubation using the following formula [10]:

$$\text{Apparent biohydrogenation (\%)} = [100 \times [(\text{CFA})_{\text{I}} - (\text{CFA})_{\text{f}}/(\text{CFA})_{\text{i}}]$$

where,

$(\text{CFA})_{\text{i}}$ = % concentration of unsaturated fatty acid at 0 h incubation
$(\text{CFA})_{\text{f}}$ = % concentration of unsaturated fatty acid at 72 h incubation

### *2.6. Statistical Analysis*

The chemical composition and secondary compound metabolism of samples were analyzed using the simple mean, while other data were subjected to a one-way analysis of variance (ANOVA) of SAS, (9.4). Mean differences were accepted as significant at ($p < 0.05$) and separated by Tukey's test. The plots of residuals against expected values were used to assess the homogeneity of variances. The data were subjected to an analysis of variance by using the statistical model:

$$Yij = \mu + si + eij$$

in which *Yij* = dependent variable; $\mu$ = overall mean; *si* = the fixed effect for supplementation of the level of corn (5 and 10%); and *eij* = experimental error that was assumed in the segments.

## 3. Results

### *3.1. Evacuation and Fermentations of Rumen*

Major gastrointestinal tract (GIT) weight differences were also recorded among the three treatment groups (Table 3). Lambs fed on 5% corn (C1%) had heavier abomasum weights compared to the C0% corn group (base diet) ($p < 0.001$) while those fed on C1% and C2% had the lowest intestine weight compared with those on base diet, which had a high value ($p < 0.05$). No significant changes in the rumen, omasum, and reticulum were observed in lambs provided with the dietary corn at any of the administered levels.

**Table 3.** Effect of different levels of corn on weight of component of full and empty gastrointestinal tract.

| Parameters | C0% | C1% | C2% | SEM | *p*-Value |
|---|---|---|---|---|---|
| Rumen full (kg) | 5.30 | 4.96 | 5.16 | 0.15 | 0.18 |
| Rumen empty (kg) | 0.66 | 0.80 | 0.87 | 0.05 | 0.43 |
| Omasum & Reticulum full (g) | 100.00 | 116.67 | 113.33 | 13.54 | 0.89 |
| Omasum & Reticulum empty (g) | 100.0 | 100.0 | 100.0 | 0.01 | 0.93 |
| Abomasum full (g) | 180.00 $^{b}$ | 293.33 $^{a}$ | 230.00 $^{b}$ | 18.02 | 0.001 |
| Abomasum empty (g) | 170.00 $^{b}$ | 200.00 $^{ab}$ | 210.67 $^{a}$ | 8.83 | 0.05 |
| Intestine full (kg) | 1.30 $^{a}$ | 1.18 $^{b}$ | 1.33 $^{ab}$ | 0.03 | 0.05 |
| Intestine empty (kg) | 0.86 $^{b}$ | 1.11 $^{ab}$ | 1.21 $^{a}$ | 0.09 | 0.05 |

C0%: (75.3% PKC + 0% corn), C1%: (70.3% PKC + 5% corn), C2%: (65.3% PKC + 10% corn). $^{a,b}$ Means in the same row with different superscripts are significantly different.

No significant differences ($p > 0.05$) were observed in IVDMD, IVOMD, and $NH_3$-N and pH at 72 h. However, a significant effect of $CH_4$ was observed, with C0% showing the highest $CH_4$ concentration when compared with C1% and C2% (Table 4).

**Table 4.** Effect of variations among treatment diets on some fermentation characteristics.

| Parameters | C0% | C1% | C2% | SEM | *p*-Value |
|---|---|---|---|---|---|
| IVDMD | 51.78 | 44.37 | 49.91 | 1.25 | 0.46 |
| IVOMD | 80.54 | 85.04 | 82.97 | 1.82 | 0.61 |
| $CH_4$ (mmol/L) | 3.36 [a] | 2.95 [b] | 2.81 [b] | 0.16 | 0.01 |
| $NH_3$-N (mg/dL) | | | | | |
| 0 h | 23.76 | 23.86 | 25.46 | 1.98 | 0.67 |
| 72 h | 36.79 | 35.69 | 32.79 | 1.78 | 0.95 |
| pH | | | | | |
| 0 h | 6.80 | 6.69 | 6.75 | 0.02 | 0.34 |
| 72 h | 6.83 | 6.79 | 6.81 | 0.01 | 0.67 |

C0%: (75.3% PKC + 0% corn), C1%: (70.3% PKC + 5% corn), C2%: (65.3% PKC + 10% corn), [a,b] Means in the same row with different superscripts are significantly different.

### 3.2. Volatile Fatty Acid

Table 5 showed the quantum of each VFA product (acetate, propionate and butyrate) before (0 h) and after (72 h) in vitro incubation. No significant difference was observed in overall VFA at 0 and 72 h. However, at 72 h of incubation only butyrate showed a significant difference ($p < 0.05$) when corn was used as the energy source. The results showed higher propionate and butyrate recorded in C2% at 72 h of incubation as compared to C0% and C1%. Furthermore, no significant differences were observed among treatments in acetate: propionate (C2: C3) in the incubation period.

**Table 5.** Concentration of volatile fatty acids (VFA, mmol/ml) after 72 h in vitro fermentation of different levels of corn substitution.

| Parameter | C0% | C1% | C2% | SEM | *p*-Value |
|---|---|---|---|---|---|
| Total VFA | | | | | |
| 0 h | 12.77 | 12.87 | 12.80 | 0.38 | 0.42 |
| 72 h | 13.61 | 14.67 | 16.86 | 0.51 | 0.51 |
| Acetate | | | | | |
| 0 h | 6.11 | 6.19 | 6.50 | 0.16 | 0.46 |
| 72 h | 7.20 | 6.48 | 7.99 | 0.04 | 0.51 |
| Propionate | | | | | |
| 0 h | 3.56 | 4.05 | 4.23 | 0.67 | 0.59 |
| 72 h | 2.94 [b] | 3.12 [ab] | 4.53 [a] | 0.31 | 0.05 |
| Butyrate | | | | | |
| 0 h | 3.36 | 3.62 | 3.47 | 0.68 | 0.93 |
| 72 h | 2.22 [b] | 2.62 [ab] | 3.52 [a] | 0.22 | 0.05 |
| C2: C3 | | | | | |
| 0 h | 1.68 | 1.74 | 1.78 | 0.10 | 0.54 |
| 72 h | 2.06 | 1.75 | 2.06 | 0.07 | 0.20 |

C0%: (75.3% PKC + 0% corn), C1%: (70.3% PKC + 5% corn), C2%: (65.3% PKC + 10% corn), [a,b] Means in the same row with different superscripts are significantly different.

### 3.3. The Rumen Microbial Profile

The effect of treatment on total bacteria population in the rumen liquor measured at different hours of in vitro experiment was presented in Table 6. However, no significant differences were observed in the total bacteria population after 24 h of incubation. The population of cellulolytic bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*) was not significantly different among the dietary treatments.

Furthermore, the *F. succinogenes* showed a reduction pattern at 24 h for C1% and C2% than C0% as the trial progressed. However, the population of *R. albus* and *R. flavefaciens* remained unchanged in all treatments during the entire period of the experiment. In this study, the methanogenic archaea population gradually reduced in the rumen liquor. At 24 h of incubation, the total methanogenic archaea increased ($p < 0.001$) in the rumen liquor by as much as 5.56 $\times 10^9$/mL in C0% while C1% remained unchanged with 4.12 $\times$ $10^9$/mL. The results showed that the mean concentration of protozoa in ruminal fluid was significantly ($p < 0.001$) higher at 24 h in C1% than in the other treatments. The average number of protozoa was significantly ($p < 0.001$) affected by the treatments (4.37, 5.72 and 5.87 $\times$ $10^5$/mL respectively for C0%, C1% and C2%) for 24 h of incubation. The protozoa population in the rumen liquor slightly increased at 24 h in all treatments except for C0% where it recorded the lowest population compared with the rest of the time in the treatments.

**Table 6.** The effect of dietary treatments of sampling on microbial population (copies/ mL) in vitro trial.

| **Item** | **Diets** | | | | |
|---|---|---|---|---|---|
| **Species** | **C0%** | **C1%** | **C2%** | **SEM** | ***p*-Value** |
| Total bacteria ($\times 10^{10}$) 24 h | 11.58 | 11.60 | 11.29 | 0.06 | 0.08 |
| *F. succinogenes* ($\times 10^9$) 24 h | 7.75 | 7.01 | 7.50 | 0.16 | 0.46 |
| *R. albus* ($\times 10^6$) 24 h | 7.86 | 6.77 | 7.78 | 0.30 | 0.27 |
| *R. flavefaciens* ($\times 10^7$) 24 h | 6.84 | 7.46 | 6.91 | 0.14 | 0.17 |
| Methanogenic archaea ($\times 10^9$) 24 h | 5.56 $^a$ | 4.12 $^b$ | 5.12 $^a$ | 0.18 | 0.001 |
| Total protozoa ($\times 10^5$) 24 h | 4.37 $^b$ | 5.72 $^a$ | 5.87 $^a$ | 0.24 | 0.001 |

C0%: (75.3% PKC + 0% corn), C1%: (70.3% PKC + 5% corn), C2%: (65.3% PKC + 10% corn), $^{a,b}$ Means in the same row with different superscripts are significantly different.

### *3.4. Fatty Acid Composition of Rumen Liquor*

The effects of corn on the fatty acid profile in the rumen fluid of slaughtered lambs are presented in Table 7. The corn diet affected ($p < 0.05$) the level of C15:0, C18:1 t-11, and Σ Trans FA. The corn substitution increased the concentration of C15:0, C18:1 t-11, and Σ Trans FA at levels C1% and C2% but decreased that of C0%. Further, the diets did not influence ($p > 0.05$) the other fatty acids detected. From the in vivo results of fatty acid composition, palmitic acid (C16:0) was the most abundant ruminal FA due to ruminal biohydrogenation, and its proportion reduced as the level of corn in the diet increased. On the other hand, the reduction of C16:0 levels may be due to a higher concentration of C16:1n-9 in the diet.

Table 8 shows the fatty acid profile of rumen liquor after 72 h of incubation. The addition of corn significantly ($p < 0.05$) increased the proportion of medium chain FA (C12:0, C15:1, C16:0). It also significantly ($p < 0.01$) increased the percentage of C18:1*trans*-11, C18:1n-9 but reduced the percentages ($p < 0.05$) of C18:0, C20:4n-6 and C22:5n-3. The ability of corn to grow Σ *Trans* FA was more ($p < 0.05$) noticeable in high concentrate substrates. However, small substitutions of substrates had significantly ($p < 0.05$) higher biohydrogenation intermediates at C0%. Apparent biohydrogenation of C18:2n-6 and C18:3n-3 was not as affected as the level of corn substituted in the substrate. Corn reduced ($p < 0.001$) the apparent biohydrogenation of C18:1n-9 in C1% and C2% compared with C0%.

**Table 7.** Fatty acid composition (g/100 g total fatty acids) of rumen fluid in vivo fermentation of different levels of corn substitution.

| Fatty Acids | C0% | C1% | C2% | SEM | *p*-Value |
|---|---|---|---|---|---|
| C12:0 | 3.79 | 3.97 | 3.80 | 0.16 | 0.91 |
| C14:0 | 3.94 | 3.97 | 4.15 | 0.15 | 0.86 |
| C15:0 | 0.36 $^{b}$ | 0.52 $^{ab}$ | 0.64 $^{a}$ | 0.05 | 0.05 |
| C15:1 | 0.44 | 0.59 | 0.58 | 0.04 | 0.27 |
| C16:0 | 40.72 | 37.34 | 35.63 | 1.02 | 0.10 |
| C16:1n-7 | 0.35 | 0.39 | 0.23 | 0.03 | 0.12 |
| C16:1n-9 | 0.20 | 0.19 | 0.38 | 0.04 | 0.15 |
| C18:0 | 33.10 | 32.21 | 36.70 | 0.93 | 0.09 |
| C18:1 c 9 | 10.01 | 11.54 | 10.04 | 0.59 | 0.54 |
| C18:1 *t*-11 | 1.33 $^{b}$ | 3.17 $^{a}$ | 2.09 $^{ab}$ | 0.28 | 0.05 |
| C18:2n-6 | 2.01 | 1.78 | 2.39 | 0.19 | 0.50 |
| CLAc9 *t*-11 | 0.85 | 0.74 | 0.94 | 0.06 | 0.52 |
| CLAc12 *t*-10 | 0.56 | 0.79 | 0.68 | 0.07 | 0.53 |
| C18:3n-3 | 1.54 | 1.28 | 1.20 | 0.09 | 0.37 |
| C20:4n-6 | 0.32 | 0.29 | 0.31 | 0.01 | 0.86 |
| C20:5n-3 | 0.09 | 0.52 | 0.12 | 0.09 | 0.09 |
| C22:5n-3 | 0.53 | 0.45 | 0.51 | 0.02 | 0.30 |
| C22:6n-3 | 0.24 | 0.23 | 0.23 | 0.01 | 0.93 |
| ΣSFA | 81.93 | 78.02 | 80.94 | 0.96 | 0.25 |
| ΣUFA | 18.07 | 21.97 | 19.05 | 0.96 | 0.25 |
| ΣMUFA | 12.33 | 13.32 | 16.45 | 0.85 | 0.10 |
| ΣPUFA n-3 | 2.42 | 2.49 | 2.07 | 0.12 | 0.37 |
| ΣPUFA n-6 | 2.33 | 2.08 | 2.60 | 0.20 | 0.65 |
| ΣPUFA | 4.75 | 4.57 | 4.67 | 0.27 | 0.97 |
| Σ *Trans* FA | 1.33 $^{c}$ | 3.17 $^{a}$ | 2.09 $^{b}$ | 0.28 | 0.05 |
| ΣCLA | 1.41 | 1.54 | 1.63 | 0.12 | 0.80 |
| n-6:n-3 | 0.98 | 0.81 | 1.24 | 0.09 | 0.14 |
| UFA:SFA | 0.22 | 0.28 | 0.23 | 0.01 | 0.22 |
| PUFA:SFA Ratio | 0.06 | 0.05 | 0.05 | 0.004 | 0.95 |

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids. $^{a,b}$ Means in the same row with different superscripts are significantly different.

**Table 8.** Fatty acid composition (g/100 g total fatty acids) of rumen fluid and rate of biohydrogenation at 72 h incubation.

| Fatty Acids | C0% | C1% | C2% | SEM | *p*-Value |
|---|---|---|---|---|---|
| C12:0 | 3.53 $^{a}$ | 2.24 $^{b}$ | 3.05 $^{a}$ | 0.24 | 0.05 |
| C14:0 | 2.62 | 2.74 | 2.97 | 0.07 | 0.12 |
| C15:0 | 2.51 | 2.81 | 2.94 | 0.11 | 0.34 |
| C15:1 | 0.91 $^{b}$ | 1.24 $^{a}$ | 1.19 $^{a}$ | 0.06 | 0.05 |
| C16:0 | 16.96 $^{b}$ | 24.34 $^{a}$ | 23.56 $^{a}$ | 1.49 | 0.001 |
| C16:1n-7 | 1.17 | 1.44 | 0.79 | 0.18 | 0.46 |
| C16:1n-9 | 1.16 | 0.60 | 1.14 | 0.16 | 0.34 |
| C18:0 | 49.64 $^{a}$ | 39.68 $^{b}$ | 40.20 $^{b}$ | 2.19 | 0.05 |
| C18:1 c 9 | 3.32 $^{b}$ | 4.91 $^{a}$ | 4.73 $^{a}$ | 0.32 | 0.001 |
| C18:1 *t*-11 | 1.89 $^{b}$ | 3.05 $^{a}$ | 2.77 $^{a}$ | 0.23 | 0.05 |
| C18:2n-6 | 10.27 | 12.56 | 10.91 | 0.66 | 0.43 |
| CLAc9 *t*-11 | 1.39 | 1.12 | 1.39 | 0.18 | 0.85 |
| CLAc12 *t*-10 | 0.81 | 1.11 | 0.91 | 0.10 | 0.59 |
| C18:3n-3 | 1.23 | 0.93 | 1.53 | 0.23 | 0.69 |
| C20:4n-6 | 0.60 $^{b}$ | 0.90 $^{a}$ | 0.59 $^{b}$ | 0.07 | 0.07 |
| C20:5n-3 | 1.40 | 0.55 | 1.21 | 0.21 | 0.27 |
| C22:5n-3 | 0.55 $^{a}$ | 0.30 $^{b}$ | 0.28 $^{b}$ | 0.05 | 0.05 |
| C22:6n-3 | 0.91 | 0.69 | 0.99 | 0.08 | 0.44 |
| ΣSFA | 75.26 | 71.83 | 72.72 | 0.93 | 0.37 |
| ΣUFA | 24.73 | 28.16 | 27.27 | 0.93 | 0.37 |

**Table 8.** *Cont.*

| Fatty Acids | C0% | C1% | C2% | SEM | *p*-Value |
|---|---|---|---|---|---|
| ΣMUFA | 8.46 [b] | 11.24 [a] | 10.64 [a] | 0.54 | 0.01 |
| ΣPUFA n-3 | 4.10 | 2.48 | 4.02 | 0.37 | 0.10 |
| ΣPUFA n-6 | 10.87 | 13.46 | 11.50 | 0.72 | 0.39 |
| ΣPUFA | 14.97 | 15.94 | 15.52 | 0.60 | 0.87 |
| Σ *Trans* FA | 1.89 [b] | 3.05 [a] | 2.77 [a] | 0.23 | 0.05 |
| ΣCLA | 2.20 | 2.23 | 2.31 | 0.20 | 0.98 |
| n-6:n-3 | 2.63 [b] | 5.43 [a] | 2.94 [b] | 0.59 | 0.05 |
| UFA:SFA | 0.33 | 0.39 | 0.37 | 0.01 | 0.35 |
| PUFA: SFA Ratio | 0.20 | 0.22 | 0.21 | 0.009 | 0.69 |
| | Apparent biohydrogenation (%) | | | | |
| C18:1n9 | 87.01 [a] | 80.79 [b] | 81.50 [b] | 1.25 | 0.001 |
| C18:2n-6c | 47.73 | 36.05 | 44.44 | 3.36 | 0.43 |
| C18:3n-3 | 63.65 | 72.63 | 54.82 | 4.17 | 0.23 |

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids. [a,b] Means in the same row with different superscripts are significantly different.

## 4. Discussion

### *4.1. Evacuation and Fermentations of Rumen*

This is while noting that the inclusion of PKC in the diet caused a certain degree of feed restriction, thereby lowering the weight of the primary metabolic organs such as the liver, heart, and small and large intestines [16]. The full intestine was lighter in lambs fed on the C1% and C2% diets than in other treatments (Table 3). An increase in rumen and intestine capacity is associated with a higher grass and roughage intake, and consumption enhances together with GIT weight increase [17]. Furthermore, the larger organ size could result in higher caloric requirements [18]. The results of GIT in the present study are in agreement with Abdelrahman et al. [19] in sheep and Buranakarl et al. [20] in goats fed a PKC diet. However, no difference was observed for the weights of the rumen and omasum-reticulum, possibly due to the chemical composition of PKC, which was used as a replacement for concentrates, perhaps, PKC indigestibility resulted in a rumen fill effect [16]. The present study found that lambs fed on a low level of corn diet were lighter in empty body weight and attributed this to the effect of gut fill caused by such a diet. In the previous study, the use of sugarcane stalks in the diet of cattle conducted by Kawashima et al. [21] revealed that harder fiber of the stalk might remain in the rumen for a relatively extended period, which would dampen consumption.

The amount of gas produced during in vitro incubation could reflect the degree of degradability and fermentation of a substrate. Substitution of PKC by corn had no effect on IVDMD, IVOMD, pH, and $NH_3$-N. The present finding is consistent with Chanjula, Mesang and Pongprayoon [4] who reported that an inclusion of up to 30% PKC in ruminants' diets did not adversely affect rumen fermentation characteristics or microbial populations. This was probably due to the inability of a microorganism to utilize the nutrients bound to the structural components of PKC under the Maillard reaction that occurred during processing of oil extraction [22]. Ruminal $NH_3$-N and pH at 0 and 72 h of incubation were not altered by diets containing corn with PKC diets, ranging from 31–36 mg/dL and pH 6.69–6.79 at 72 h. The level of ruminal $NH_3$-N exceeded 5–8 mg/dL in all treatment groups, which is the optimal level of $NH_3$-N for microbial protein synthesis [23,24], while all treatment pH means were also within the normal range, with the values showing relative stability at 6.22–6.53, which is the optimal level for microbial digestion of fiber and protein at 6.0–7.0 [4,25,26]. The rumen $NH_3$-N concentration has not differed, but the observed value of $NH_3$-N was slightly higher in C0% and C1%. This was a relatively greater concentration of rumen $NH_3$-N and agreed with Satter and Slyter [23], who reported 5 mg/dL as minimum level of rumen $NH_3$-N for optimum microbial protein synthesis. The higher level of $NH_3$-N observed in this study may probably be due to the high proportion

of non-protein nitrogen (NPN) in the rice straw. The decline in $NH_3$-N concentration might be due to more efficient N-utilization by rumen microbes when fermentable energy was available. As shown in the present study, dietary components such as NDF and ADF also contribute to the difference in $CH_4$ production. High levels of NDF raise $CH_4$ production by moving the short-chain fatty acid fraction towards acetate which is responsible for producing more hydrogen. The type of carbohydrate present in the diet is thought to dictate $CH_4$ production via changes in the ruminal microbial [27–29].

### *4.2. Volatile Fatty Acid*

Fermentation in the rumen produces volatile fatty acids, such as acetate, propionate, and butyrate acids, which can be metabolized by the animal. In the present study, the means of total VFA, acetate, propionate, and butyrate concentrations in the rumen were unaffected by dietary treatments, but in terms of the numbers and the overall level of VFA, they were a little lower in C0% when compared with other treatments, most likely because of the low apparent digestibility. In the present study, there was a continuous decrease in propionate and butyrate production, but no difference in acetate after 72 h, which may be due in the presence of rice straw, which is high in dietary fiber. Treatment groups substituted with 5% and 10% corn (C1% and C2%) showed a significant increase in propionate at 72 h. Generally, in ruminants, rapidly fermentable substrates have a relatively higher production of propionate acid. On the other hand, slowly fermentable and cellulose-rich substrates will have high acetate acid-directed fermentation products. High concentrations of soluble carbohydrate promote propionate production in the rumen, and a lower ruminal pH inhibits methanogen growth, thereby reducing $CH_4$ production per unit of fermented organic matter. This is in agreement with Atasoy et al. [30] who reported that VFA produced in the rumen is affected by several factors such as pH, feed composition, and microbial species. However, Dias [31] reported that higher levels of PKC in the diet of goats may probably reduce the digestibility of energy from PKC, mainly because of the low content of starch. Butyrate production was significantly higher in C2% than the control at 72 h of incubation and this could be as a result of ruminal protozoa dominance that produces butyrate acid as their end product of carbohydrate fermentation [14,32]. The acetate-propionate ratio was the same across dietary treatments in the present study, as reported by Zhang et al. [33].

### *4.3. Rumen Microbial Profile*

In recent years, there has been an increasing amount of literature on dietary corn substitution effects on total bacteria populations in the rumen [14]. The total bacteria population in C1% and C2% declined after 24 h. This may be due to the increase in the protozoa and their engulfing effect on the rumen bacteria [34]. This is in contrast to the findings of Abubakr et al. [35], who attributed the increase in rumen bacteria population in goats fed a PKC-based diet to the rapid multiplication after elimination of protozoa. The reason for this was not clear but it could be due to bacterial predation by rumen protozoa which is mainly reliant on the size and type of protozoa such as the holotrich protozoa, which has much lower predatory activity than entodiniomorphids [36]. Moreover, the mean of rumen total bacteria population in these in vitro trials had significantly reduced in C0%; it is well established that fatty acids composition in substrate could be toxic to total bacteria [32], while long or short-term defaunation may reduce the rumen bacteria population. Methanogens archaea respond differently to different levels of corn at 24 h. In our experiment, the relative abundance of methanogens archaea, slightly increased in response to corn in C2%, was reported to be affected slightly [37]. Methanogenesis often utilizes the resultant hydrogen and $CO_2$ formed during the fermentation of carbohydrates as VFA. Therefore, eliminating hydrogen from the ruminal environment during carbohydrate fermentation could allow methanogens to permit the active fermentation microorganisms to function at an optimal rate with the resultant formation of complete oxidation of substrates [38]. With this, therefore, increasing methanogens archaea from

the higher degradability of feed in C2% at 24 h with the releases of $H_2$ subsequently may enhance the ability of methanogens archaea to grow. Johnson and Johnson [27] reported that the digestion of cell wall fiber enhances $CH_4$ production thereby raising the quantum of acetate production and lowering the propionate produced. The rise in $CH_4$ emissions is due to the fermentation of acetate, which creates a methyl group for methanogenesis. The level of methanogens observed at C1% is an indication of low $CH_4$ emission, since the hydrogen ions needed by methanogens to reduce $CO_2$ to $CH_4$ were drastically reduced [39]. As a component of medium-chain fatty acids, it can potentially hamper rumen methanogenesis and methanogens because of its toxic effect. Rumen protozoa are in control of the symbiotic transfers of $H_2$ to methanogens, which are utilized to increase $CH_4$ and produce over 25% of $CH_4$ in the rumen [40]. Meanwhile, Machmüller et al. [41] reported that the relationship between protozoa and methanogens does not play a significant role in rumen methanogenesis. Changes in the proportion of the different methanogen species are due to a lower $O_2$ pressure and greater $H_2$ availability within the protozoal cells [32]. Rumen protozoa were affected by treatments at 24 h incubation, and overall protozoal populations were increased by the substitution of PKC with 5% and 10% of corn as sources of energy in the treatment (C1% and C2%), perhaps as a result of a higher level of corn in the diets which enhanced the dominance of protozoa over the rest of the rumen microbial population. This finding was in agreement with Abdullah and Hutagalung [42], who reported a negative influence of PKC on rumen protozoa of cattle fed a PKC-based diet. Some dietary factors may decrease or even remove ruminal protozoa. In addition, Abdullah et al. [43] reported that the number of protozoa in the rumen fluid of sheep was reduced after consuming PKC in the first two groups of sheep. The reduction in rumen protozoa population numbers may be attributed to lower butyrate production. It has also been reported that unsaturated fatty acids reduce protozoa numbers [44] due to the fact that unsaturated C18 fatty acids are toxic to protozoa. Therefore, the use of PKC-based diets may have the potential to reduce protozoa numbers, thereby changing the ruminal ecosystem, and indirectly increasing the bacterial population and activity. However, because the protozoa and, hence, the production of sulfide, decrease the bioavailability of dietary Cu, our findings show that protozoa levels are raised at higher levels of corn as an energy source, which is deemed a motivator for protozoa growth [14].

### *4.4. Fatty Acid Profile of Rumen*

The presence of C15:0 in the diet contrasts with previous studies conducted by Wu et al. (1991) and Adeyemi et al. (2015b) who reported that, although C15:0 was absent from the diets, the abundance of C15:0 detected in the ruminal digesta suggested that it was created by the microbial population. Bauman et al. (2003), however, report that CLAc9t11 is only a transitory intermediate, and it is the trans-11 C18:1 that accumulates.

The corn substitute group had a higher proportion of C18:1*trans*-11 than the control group. This could be due to the ability of rumen bacteria to synthesize the biohydrogenation intermediate from C18:2n-6 [45] which is abundant in the C1% and C2% diets. Therefore, a greater proportion of C18:1*trans*-11 is expected in diets containing a higher proportion of C18:2n-6. The total *trans* FA increased with increasing levels of corn. This could be due to biohydrogenation, which yielded more C16:0 and C18:0 in the C1% and C2% compared with the control diet. The total FA increased linearly with the increase in energy, reflecting the level of dietary fat.

The main fatty acid substrate for biohydrogenation in ruminants was linolenic acid (cis-9,cis-12,cis-15-18:3) because it was the most abundant fatty acid present in glycolipids and phospholipids of feed [10,46]. The current study found that the proportion of fatty acids were not affected by corn dietary intake at 72 h; this could be due to the composition of fatty acids in these diets. A significant reduction of C18:0 could be due to incomplete biohydrogenation of C18:2n6c, C18:1n9c and C18:3n-3 which yielded a higher concentration of biohydrogenation intermediates C18:1 *t*-11 (72 h) C0%. The C18:1n9 declined significantly with increasing levels of corn for this study at 72 h. This is contrary to what we have expected and may be due to the ability of rumen bacteria to synthesize the biohydrogenation

intermediate from C18:2n-6 [47]. In the present study, palmitic (C16:0) was the second most abundant ruminal FA at 72 h although its proportion was heavily influenced by corn diets. A possible explanation for this may be that palm oils and their byproducts are considered good sources of C16:0 [48]. The control diet had a lower proportion of C18:1*trans*-11 at 72 h than other treatments. It was established that these unsaturated fatty acids include CLA and vaccenic acid [49], further increasing the possibility of significance of protozoa in the delivery of health-promoting FA from the rumen. Beam, et al. [50] reported that the extent of biohydrogenation increases in tandem with the degree of unsaturation of fatty acids. The increasing levels of substituted corn could probably be responsible for the significantly lower concentrations of C18:1n9c. It has been established that the incomplete biohydrogenation of linoleic acid [51,52], linolenic acid, and oleic acid yielded *t*-11-18:1, CLAc9 *t*11, CLAc12 *t*10 and other conjugated isomers. Protozoa play a significant role in biohydrogenation of the rumen microbial FA and contain proportionally more unsaturated fatty acids and CLA [53]. It is well-known that protozoal lipids contain proportionally more unsaturated FA than the bacterial fraction [14,53]. However, Serrapica et al. [54] indicated that due to differences in the source material, such as the presence of gelatinous compounds and the absence of surface area for bacteria to adhere to, bacterial adhesion is inhibited.

## 5. Conclusions

In conclusion, the inclusion of corn in the PCK basic diet at varying levels did not have an effect on the rumen's fatty acid content. However, the biohydrogenation percentage of C18:1n9 was decreased under in vitro conditions. The substitution of corn improved fermentation characteristics in vitro with no adverse effects. In addition, it had a quantitative impact on the total number of protozoa. Short-chain fatty acid absorption seems to help stabilize ruminal pH by eliminating the effect of toxic substances in the rumen.

**Author Contributions:** O.A.S. analyzed and interpreted data regarding the laboratory analysis. U.M.S. was a major contributor to the calculations. O.A.S., A.Q.S., H.A., A.R.A. and A.A.S. participated in the whole design of the study, performed the statistical analysis and contributed to the preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the Institutional Committee on Animal Use Ethics (Approval No. R064/2016) of University Putra Malaysia.

**Data Availability Statement:** Data and materials used and analyzed during this study are available from the corresponding author on reasonable request.

**Acknowledgments:** The authors extend their appreciation to Universiti Putra Malaysia (UPM) for funding this work and the Higher Education Ministry and Scientific Research of Iraq and Department of Animal Production, University of Anbar for their support. This research did not receive any specific funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Ribeiro, R.D.X.; Oliveira, R.L.; Macome, F.M.; Bagaldo, A.R.; Silva, M.C.A.D.; Ribeiro, C.V.D.M.; Carvalho, G.G.P.D.; Lanna, D.P.D. Meat Quality of Lambs Fed on Palm Kernel Meal, a By-Product of Biodiesel Production. *Asian Australas. J. Anim. Sci.* **2011**, *24*, 1399–1406. [CrossRef]
2. Rahman, M.M.; Abdullah, R.B.; Embong, W.K.W.; Nakagawa, T.; Akashi, R. Effect of palm kernel cake as protein source in a concentrate diet on intake, digestibility and live weight gain of goats fed Napier grass. *Trop. Anim. Health Prod.* **2013**, *45*, 873–878. [CrossRef] [PubMed]
3. Alimon, A. The nutritive value of palm kernel cake for animal feed. *Palm Oil Dev.* **2004**, *40*, 12–14.
4. Chanjula, P.; Mesang, A.; Pongprayoon, S. Effects of dietary inclusion of palm kernel cake on nutrient utilization, rumen fermentation characteristics and microbial populations of goats fed *Paspalum plicatulum* hay-based diet. *Sonklanakarin J. Sci. Technol.* **2010**, *32*, 527.

5. Xu, N.; Wang, D.; Wang, B.; Wang, J.; Liu, J. Different endosperm structures in wheat and corn affected in vitro rumen fermentation and nitrogen utilization of rice straw-based diet. *Animal* **2019**, *13*, 1607–1613. [CrossRef]
6. Saeed, O.A.; Sazili, A.Q.; Akit, H.; Ebrahimi, M.; Alimon, A.R.; Samsudin, A.A. Effects of corn supplementation on meat quality and fatty acid composition of Dorper lambs fed PKC-Urea treated rice straw. *BMC Veter. Res.* **2019**, *15*, 233. [CrossRef]
7. Saeed, O.A.; Kee, L.T.; Sazili, A.Q.; Akit, H.; Jahromi, M.F.; Alimon, A.R.; Samsudin, A.A. Effects of corn supplementation on the antioxidant activity, selected minerals, and gene expression of selenoprotein and metallothionein in serum, liver, and kidney of sheep-fed palm kernel cake: Urea-treated rice straw diets. *3 Biotech* **2019**, *9*, 146. [CrossRef]
8. Saeed, O.A.; Sazili, A.Q.; Akit, H.; Alimon, A.R.; Mazlan, M.; Samsudin, A.A. The Growth Efficiency and Carcass Characteristics of Dorper Sheep Treated by Corn Inclusion as Energy into Palm Kernel Cake Based-Diet. *Trop. Anim. Sci. J.* **2018**, *41*, 29–36. [CrossRef]
9. 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*, 7–55.
10. Adeyemi, K.D.; Ebrahimi, M.; Samsudin, A.A.; Alimon, A.R.; Karim, R.; Karsani, S.A.; Sazili, A.Q. Influence of Carotino oil on in vitro rumen fermentation, metabolism and apparent biohydrogenation of fatty acids. *Anim. Sci. J.* **2015**, *86*, 270–278. [CrossRef]
11. Parsons, T.; Maita, Y.; Lalli, C.M. *Amanual of Chemical and Biological Methods for Seawater Analysis*; Pergamon: Oxford, UK, 1984; Volume 1, p. 173.
12. Cottyn, B.G.; Boucque, C.V. Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid. *J. Agric. Food Chem.* **1968**, *16*, 105–107. [CrossRef]
13. Moss, A.R.; Jouany, J.-P.; Newbold, J. Methane production by ruminants: Its contribution to global warming. *Ann. Zootech.* **2000**, *49*, 231–253. [CrossRef]
14. Saeed, O.A.; Sazili, A.Q.; Akit, H.; Alimon, A.R.; Samsudin, A.A.B. Effect of corn supplementation on purine derivatives and rumen fermentation in sheep fed PKC and urea-treated rice straw. *Trop. Anim. Health Prod.* **2018**, *50*, 1859–1864. [CrossRef] [PubMed]
15. Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
16. Dos Santos, R.D.C.; Gomes, D.I.; Alves, K.S.; Mezzomo, R.; Oliveira, L.R.S.; Cutrim, D.O.; Sacramento, S.B.M.; de Moura Lima, E.; de Carvalho, F.F.R. Carcass characteristics and meat quality of lambs that are fed diets with palm kernel cake. *Asian Australas. J. Anim. Sci.* **2017**, *30*, 865–871. [CrossRef]
17. De Oliveira, A.A.; Romanzini, E.P.; Costa, D.F.A.; Barbero, R.P.; Azenha, M.V.; Lage, J.F.; Ruggieri, A.C.; Reis, R.A. Citrus pulp replacing corn in the supplement decreased fibre digestibility with no impacts on performance of cattle grazing marandu palisade grass in the wet-dry transition period. *Animals* **2022**, *12*, 822. [CrossRef]
18. Xie, G.; Li, Z.; Ran, Q.; Wang, H.; Zhang, J. Over-expression of mutated *ZmDA 1 or ZmDAR 1* gene improves maize kernel yield by enhancing starch synthesis. *Plant Biotechnol. J.* **2018**, *16*, 234–244. [CrossRef]
19. Abdelrahman, M.M.; Alhidary, I.; Albaadani, H.H.; Alobre, M.; Khan, R.U.; Aljumaah, R.S. Effect of Palm Kernel Meal and Malic Acid on Rumen Characteristics of Growing Naemi Lambs Fed Total Mixed Ration. *Animals* **2019**, *9*, 408. [CrossRef]
20. Buranakarl, C.; Thammacharoen, S.; Semsirmboon, S.; Sutayatram, S.; Chanpongsang, S.; Chaiyabutr, N.; Katoh, K. Effects of replacement of para-grass with oil palm compounds on body weight, food intake, nutrient digestibility, rumen functions and blood parameters in goats. *Asian Australas. J. Anim. Sci.* **2020**, *33*, 921–929. [CrossRef]
21. Kawashima, T.; Sumamal, W.; Pholsen, P.; Chaithiang, R.; Hayashi, Y. Ruminal Degradation of Sugarcane Stalk. *Asian Australas J. Anim. Sci.* **2003**, *16*, 1280–1284. [CrossRef]
22. Berk, E.; Hamzalıoğlu, A.l.; Gökmen, V. Investigations on the Maillard reaction in sesame (*Sesamum indicum* L.) seeds induced by roasting. *J. Agric. Food Chem.* **2019**, *67*, 4923–4930. [CrossRef] [PubMed]
23. Satter, L.D.; Slyter, L.L. Effect of ammonia concentration on rumen microbial protein production in vitro. *Br. J. Nutr.* **1974**, *32*, 199–208. [CrossRef] [PubMed]
24. Mamuad, L.L.; Kim, S.H.; Biswas, A.A.; Yu, Z.; Cho, K.-K.; Kim, S.-B.; Lee, K.; Lee, S.S. Rumen fermentation and microbial community composition influenced by live *Enterococcus faecium* supplementation. *Amb. Express* **2019**, *9*, 123. [CrossRef] [PubMed]
25. Van Soest, P. *Nutritional Ecology of the Ruminant*, 2nd ed.; Cornell University Press: New York, NY, USA, 1994.
26. Gao, R.; Luo, Y.; Xu, S.; Wang, M.; Sun, Z.; Wang, L.; Yu, Z. Effects of replacing ensiled-alfalfa with fresh-alfalfa on dynamic fermentation characteristics, chemical compositions, and protein fractions in fermented total mixed ration with different additives. *Animals* **2021**, *11*, 572. [CrossRef] [PubMed]
27. Johnson, K.A.; Johnson, D.E. Methane emissions from cattle. *J. Anim. Sci.* **1995**, *73*, 2483–2492. [CrossRef] [PubMed]
28. Newbold, C.J.; Ramos-Morales, E. Review: Ruminal microbiome and microbial metabolome: Effects of diet and ruminant host. *Animal* **2020**, *14*, s78–s86. [CrossRef]
29. 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]
30. Atasoy, M.; Eyice, O.; Schnürer, A.; Cetecioglu, Z. Volatile fatty acids production via mixed culture fermentation: Revealing the link between pH, inoculum type and bacterial composition. *Bioresour. Technol.* **2019**, *292*, 121889. [CrossRef]

31. Dias, F.N. Supplementation of palm kernel expeller to grazing dairy farms in New Zealand: A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Science at Massey University, Palmerston North, New Zealand. Ph.D. Thesis, Massey University, Palmerston North, New Zealand, 2010.
32. Williams, A.; Coleman, G. The rumen protozoa. In *The Rumen Microbial Ecosystem*; Springer: Berlin/Heidelberg, Germany, 1997; pp. 73–139.
33. Zhang, C.; Li, M.; Al-Marashdeh, O.; Gan, L.; Zhang, C.; Zhang, G. Performance, rumen fermentation, and gastrointestinal microflora of lambs fed pelleted or unpelleted total mixed ration. *Anim. Feed Sci. Technol.* **2019**, *253*, 22–31. [CrossRef]
34. Majewska, M.P.; Miltko, R.; Bełżecki, G.; Kędzierska, A.; Kowalik, B. Protozoa population and carbohydrate fermentation in sheep fed diet with different plant additives. *Anim. Biosci.* **2021**, *34*, 1146–1156. [CrossRef]
35. Abubakr, A.; Alimon, A.R.; Yaakub, H.; Abdullah, N.; Ivan, M. Effect of Feeding Palm Oil By-Products Based Diets on Total Bacteria, Cellulolytic Bacteria and Methanogenic Archaea in the Rumen of Goats. *PLoS ONE* **2014**, *9*, e95713. [CrossRef] [PubMed]
36. Francisco, A.E.; Santos-Silva, J.M.; Portugal, A.P.V.; Alves, S.P.; Bessa, R.J.B. Relationship between rumen ciliate protozoa and biohydrogenation fatty acid profile in rumen and meat of lambs. *PLoS ONE* **2019**, *14*, e0221996. [CrossRef] [PubMed]
37. Lu, Y.; Zhang, Q.; Wang, X.; Zhong, H.; Zhu, J. Effects of initial microbial community structure on the performance of solid-state anaerobic digestion of corn stover. *J. Clean. Prod.* **2020**, *260*, 121007. [CrossRef]
38. Fu, S.-F.; Liu, R.; Sun, W.-X.; Zhu, R.; Zou, H.; Zheng, Y.; Wang, Z.-Y. Enhancing energy recovery from corn straw via two-stage anaerobic digestion with stepwise microaerobic hydrogen fermentation and methanogenesis. *J. Clean. Prod.* **2020**, *247*, 119651. [CrossRef]
39. Bhatta, R.; Saravanan, M.; Baruah, L.; Prasad, C. Effects of graded levels of tannin-containing tropical tree leaves on in vitro rumen fermentation, total protozoa and methane production. *J. Appl. Microbiol.* **2015**, *118*, 557–564. [CrossRef]
40. Roque, B.M.; Brooke, C.G.; Ladau, J.; Polley, T.; Marsh, L.J.; Najafi, N.; Pandey, P.; Singh, L.; Kinley, R.; Salwen, J.K. Effect of the macroalgae *Asparagopsis taxiformis* on methane production and rumen microbiome assemblage. *Anim. Microbiome* **2019**, *1*, 3. [CrossRef]
41. Machmüller, A.; Soliva, C.R.; Kreuzer, M. Methane-suppressing effect of myristic acid in sheep as affected by dietary calcium and forage proportion. *Br. J. Nutr.* **2003**, *90*, 529–540. [CrossRef]
42. Abdullah, N.; Hutagalung, R. Rumen fermentation, urease activity and performance of cattle given palm kernel cake-based diet. *Anim. Feed Sci. Technol.* **1988**, *20*, 79–86. [CrossRef]
43. Abdullah, N.; Hanita, H.; Ho, Y.; Kudo, H.; Jalaludin, S.; Ivan, M. The effects of bentonite on rumen protozoal population and rumen fluid characteristics of sheep fed palm kernel cake. *Asian Australas. J. Anim. Sci.* **1995**, *8*, 249–254. [CrossRef]
44. Machmüller, A.; Kreuzer, M. Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. *Can. J. Anim. Sci.* **1999**, *79*, 65–72. [CrossRef]
45. Conte, G.; Dimauro, C.; Serra, A.; Macciotta, N.P.P.; Mele, M. A canonical discriminant analysis to study the association between milk fatty acids of ruminal origin and milk fat depression in dairy cows. *J. Dairy Sci.* **2018**, *101*, 6497–6510. [CrossRef]
46. Prasad, P.; Savyasachi, S.; Reddy, L.P.A.; Sreedhar, R.V. Physico-chemical Characterization, Profiling of Total Lipids and Triacylglycerol Molecular Species of Omega-3 Fatty Acid Rich B. *arvensis Seed Oil from India. J. Oleo Sci.* **2019**, *68*, 209–223. [CrossRef]
47. Siurana, A.; Ferret, A.; Rodriguez, M.; Vlaeminck, B.; Fievez, V.; Calsamiglia, S. Strategies to modify the ruminal biohydrogenation of polyunsaturated fatty acids and the production of *trans*-10, *cis*-12 C18: 2 in vitro. *Anim. Feed Sci. Technol.* **2018**, *235*, 158–165. [CrossRef]
48. Abubakr, A.; Alimon, A.R.; Yaakub, H.; Abdullah, N.; Ivan, M. Effect of Feeding Palm Oil By-Products Based Diets on Muscle Fatty Acid Composition in Goats. *PLoS ONE* **2015**, *10*, e0119756. [CrossRef] [PubMed]
49. Devillard, E.; McIntosh, F.M.; Newbold, C.J.; Wallace, R.J. Rumen ciliate protozoa contain high concentrations of conjugated linoleic acids and vaccenic acid, yet do not hydrogenate linoleic acid or desaturate stearic acid. *Br. J. Nutr.* **2006**, *96*, 697–704. [PubMed]
50. Beam, T.; Jenkins, T.; Moate, P.; Kohn, R.; Palmquist, D. Effects of Amount and Source of Fat on the Rates of Lipolysis and Biohydrogenation of Fatty Acids in Ruminal Contents. *J. Dairy Sci.* **2000**, *83*, 2564–2573. [CrossRef]
51. Harfoot, C.; Hazlewood, G. Lipid metabolism in the rumen. In *The Rumen Microbial Ecosystem*; Hobson, P.N., Ed.; Elsevier Applied Science: London, UK, 1997; pp. 382–426.
52. Jenkins, T.C.; Wallace, R.J.; Moate, P.; Mosley, E.E. Board-Invited Review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. *J. Anim. Sci.* **2008**, *86*, 397–412. [CrossRef] [PubMed]
53. Jayanegara, A. Pattern of Polyunsaturated Fatty Acid Biohydrogenation as Influenced by Dietary Tannin. *JITV* **2014**, *19*, 8–14.
54. Serrapica, F.; Masucci, F.; Raffrenato, E.; Sannino, M.; Vastolo, A.; Barone, C.M.A.; Di Francia, A. High Fiber Cakes from Mediterranean Multipurpose Oilseeds as Protein Sources for Ruminants. *Animals* **2019**, *9*, 918. [CrossRef]