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# Effect of Mid-Term Dietary Administration of the *Caesalpinia coriaria* Extract on the Sustainable Mitigation of Equine Fecal Methane, Carbon Monoxide and Hydrogen Sulfide Production

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ABSTRACT

This study aimed to evaluate the dietary administration of the *Caesalpinia coriaria* (CC) extract for 30 days on *in vitro* fecal greenhouse gases production. Fecal samples, as inoculums, were collected from horses given daily 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 60) mL CC aqueous extract per animal. The extract dose was mixed with the morning feeding diet at 6:00 h for each horse. During incubation, 0-, 0.6-, 1.2- and 1.8-mL CC extracts were added to the basal diet which was fed to horses (as subtract) and evaluated with each fecal type. Feces from the horses given no CC extract produced the lowest ($P = .0014$) methane while the fecal from horses given CC produced more methane . It was also observed that all CC doses linearly ($P = .0457$) produced more methane than the control. Furthermore, Fecal 0 was more efficient and produced less methane for every unit of metabolizable energy, organic matter, and short chain fatty acids while Fecal 60 was the least efficient. Production of $H_2S$ showed that feces of equine orally give 60 mL/day CC produced the highest while Fecal 0 and Fecal 120 were similar. Fecal type x dose showed that 0 mL/g DM produced the highest $H_2S$ while 1.8 mL/g DM produced the lowest. Thus, based on gas production, $H_2S$, CO and $CH_4$, feeding horses with 60 mL/day of CC with or without 0.6 mL/g DM of CC extract is recommended for the sustainable mitigation of greenhouse gases emission in horses.



## 1. Introduction

There is an increasing awareness on the role of hindgut fermenters such as equine and termites on greenhouse gases (GHG) production especially methane. Methane is generated in hindgut fermenters because of the consumption of woody or high fibrous diets. As alternative to methanogenesis, acetogenesis is the form of hydrogen sink in hindgut fermenters [1] which is then used by acetogens to form acetate. Beside the concern of GHG, it is reported that the activities of equine expose them to oxidative stress which subjects them to the emergence of different pathologies [2]. As such, antioxidants such as selenium, zinc, vitamins C and E, are used in equine diets [3]. Gases of CO and $H_2S$ can also perform therapeutic activities and could be alternative means of hydrogen sinking [4,5]. The therapeutic function lies in the improvement of the antioxidative status of gastric mucosa [6] as well as the indirect reduction of methane production.

It is well established in methane producing livestock that plant extract *in vitro* and *in vivo* can mitigate the production of GHG [7,8,9,10]. These plant extracts reduced $CH_4$, $CO_2$, and $H_2$ either by inhibiting the activities of methanogens, decrease protozoa population and/or provide alternative hydrogen sink etc. Notwithstanding, there is need for continual studies on additives that could be used in diets to reduce greenhouse gasses, as well as those that produce therapeutic functions. The need to study the GHG emission mitigation strategies is due to increasing attention being paid to the methane emission from hindgut fermenters [1,11] and the possibility of their population explosion in the future which may multiply hindgut emitters output in total.

*Caesalpinia coriaria* are very rich in tannin, phenol, and flavonoids and their use in ruminants is documented [12,13], and it is widespread in the region this study was conducted [14]. How-

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☆☆ Animal welfare/ethical statement: Not applicable.

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**Table 1**
Chemical composition of the experimental diet (substrate) and the secondary metabolites of *Caesalpinia coriaria* fruit.

| Diet | Chemical Composition (% DM) |
|---|---|
| Crude protein | 13.3 |
| Nitrogen-free extract | 58.2 |
| Crude fiber | 8.0 |
| Ash | 5.0 |
| Ether extract | 3.0 |
| Secondary metabolites of *Caesalpinia coriaria* | |
| Total Phenols (mg gallic acid equivalent / g of dry extract) | 770.4 ± 0.02 |
| Total flavonoid (mg quercetin equivalent / g of dry extract) | 149.1 ± 0.03 |
| Total tannins (mg tannic acid equivalent/ g of dry extract) | 261.6 ± 0.05 |

ever, no research has been reported on the impact of *Caesalpinia coriaria* fruit on $CH_4$, CO and $H_2S$ in equine fecal biogases production. Thus, this study was conducted to evaluate the effect of mid-term dietary extract administration of the *Caesalpinia coriaria* fruit on the sustainable mitigation of equine fecal biogases production of methane, carbon monoxide and hydrogen sulfide.

## 2. Materials and Methods

### 2.1. Study and Fruit Collection

The laboratory analysis was done at the bromatology laboratory of the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of the State of Mexico. The vegetative material was collected in the state of Guerrero, and the fruits belonging to the genus and species *Caesalpinia coriaria* (CC- Cascalote), were harvested manually and randomly from different trees in July 2021. For their transfer to the bromatology laboratory, the fruits were stored and kept refrigerated at 4°C to avoid changes in their composition. Subsequently, they were dried in the shade at a temperature below 30°C and finally the fruits were processed in a semi-industrial mill (Willey Mill ) to a particle size of 3–5 mm, to be subsequently analyzed for the chemical components, secondary metabolites, and preparation of the extract to produce gas *in vitro*.

### 2.2. Preparation of the Aqueous Extract

For the preparation of the extract, the vegetative material (fruit) was ground to a particle size of 3–5 mm, then 1 kg of the ground fruit/8 L of water was extracted, and it was left to macerate for 48 to 72 hours under the shade at room temperature with regular agitation to extract the soluble substances from the Cascalote fruit. The medium was filtered through 3–4 layers of gauze and the extract was collected. The extract was prepared weekly (with a stock volume of 5 L) and this mixture was stored at 4°C and subsequently used for the daily administration of the horses. The chemical composition of the Cascalote fruit extract is shown in Table 1.

### 2.3. Animals as Fecal Inoculum Donors

To carry out this study, 12 male equines were used, in a range of 4–14 years of age with a weight of 400–550 kg body weight, consisting of 6 different breeds (Apallosa, Arabian, Aztec, Percheron 1/4 of a mile, and Spanish), for 30 days. A completely randomized design was used, and they were assigned to 3 treatment groups of *Caesalpinia coriaria extract* (i.e., CC) at 0-, 60-, and 120- mL/day for each animal (mixed with the morning feeding at 06:00 hour) during the 30 days of the experiment, with 4 horses each. The horses were fed individually a concentrated basal diet of a commercial concentrate mixture (Horse Power 1-1.2 kg/day), kernels (X-CELLENCE 40–50 g/day), bran (200–210 g/day), minerals (20 g/day), plus oat Straw (4–5 kg/day), twice a day at 06:00 and 17:00 and water available *ad libitum*. The individual fecal samples were collected from the rectum of each animal before the first evacuation of the day, during the last week. Thereafter, they were mixed and homogenized with all the samples of the group (4 horses) to obtain a representative and homogeneous sample of the fecal content per group (approximately 1/2 kg) which was used as a source of microorganisms in *in vitro* incubation.

### 2.4. In Vitro Incubations

The inoculum source of the horses groups (i.e., Fecal 0, Fecal 60 and Fecal 120) was mixed with the buffer solution of Goering and Van Soest [15] without tryptic in the ratio of 1:4 vol /vol. The incubation medium was mixed and used to inoculate 3 identical series of incubation into 120 mL serum bottles containing 0.5 g of substrate (diet: 70% forage and 30% concentrate- same diet fed to horses) in the presence of different doses of the CC extract (0-, 0.6-, 1.2- and 1.8- mL), considering as blanks the bottles with substrates but that did not contain the extract.

After filling all the bottles with the substrate, the extract, buffer solution, and the fecal content were shaken and placed in an incubator (BINDER Model FD115, Germany) at 39°C. Total gas production (psi) was recorded at 2, 4, 6, 10, 14, 21, 26, 28, 33, 37 and 48 hours following the technique of Theodorou et al. [16]. The productions of $CH_4$, CO, and $H_2S$ were also measured at same hours of incubation using a diffusion-based gas detector of 5 mL (MONITOR de Dräger Safety X-am 20500, Lübeck, Germany). After each recording, using a syringe needle, the gas was dispersed to prevent gas accumulation.

### 2.5. Apparently Degraded Substrate

The supernatant pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico), and the bottle content was filtered through sintered glass crucibles (100–160 mm; Pyrex, Stone, UK). To estimate the apparent disappearance of dry matter, the incubation residue was dried at 70°C overnight. The DM degradability at 48 hours of incubation (ie substrate degraded, DMD; %) was calculated according to [17].

### 2.6. Diet Chemical Analysis and Extract Secondary Metabolites

Proximate analysis of the diet samples (3 subsamples) was analyzed according to [18]. Fiber fraction analysis was carried out using an ANKOM200 Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY) according to AOAC [18] with the NDF and ADF done according to Rodriquez et al. [19].

#### 2.6.1. Determination of the Total Phenolic Content

Total phenolic content of the *Caesalpinia coriaria* (i.e., CC) fruits extract was evaluated by a colorimetric method utilizing Folin-Ciocalteu reagent [20]. One mL of extract was dissolved in 2 mL of methanol, 500 µL aliquots of extract were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The tubes were vortexed for 10 second and allowed to stand for 2 hours at 25°C. After incubation at 25°C for 2 hours, absorbance was measured at 765 nm against reagent blank. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram.

#### 2.6.2. Determination of Total Flavonoid Content

For the total flavonoid determination, a modified $AlCl_3$ calorimetric method was used [20]. One mL of CC fruit extract was dissolved in 2 mL of methanol in a 10 mL of volumetric flask. Solution of 5% $NaNO_3$, 5% NaOH and 7% $AlCl_3$ was prepared using water in a 25 mL of volumetric flask. 200 µL of extracts were taken in a

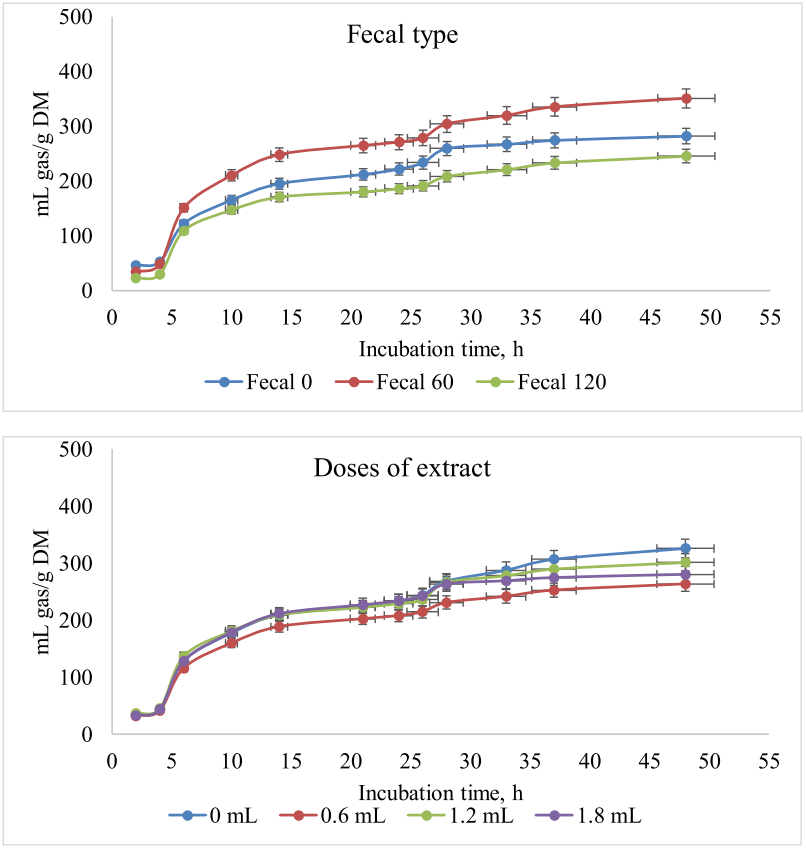


**Fig 1.** Horse total gas production (mL/g dry matter (DM)) at different hours of incubation as affected by the dietary inclusion of the accuse extract of *Caesalpinia coriaria* (Jacq.) Wild fruit at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/d/horse during 30 days of the experiment.

sealed glass vial and 75 µL of 5% $NaNO_3$ was added and left for 5 minutes at room temperature. Later, 1.25 mL of $AlCl_3$ and 0.5 mL NaOH were added to each vial. Then it was sonicated and incubated for 5 minutes at room temperature. After incubation, the absorbance was measured against methanol blank at 510 nm. The flavonoids content of extracts was estimated by using the quercetin standard calibration curve and the obtained results of flavonoids were expressed as microgram of quercetin equivalent per one gram of dry extract.

### 2.6.3. Total Condensed Tannin Contents

The total tannin contents were determined by the method described by Broadhurst and Jones [21] using tannic as a reference compound. A volume of 400 µL of extract was added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm.

## 2.7. Calculations

To estimate the kinetic parameters, gas production (GP), $CH_4$, CO, and $H_2S$ (mL/g DM) data were fitted using the NLIN option of SAS [22] to the France et al. [23] model as:

$$A = b \times \left(1 - e^{-c(t-Lag)}\right)$$

where $A$ is the volume of gases at time $t$; $b$ the asymptotic gases (mL/g DM); $c$ is the rate of gases/hour), and $Lag$ (hour) is the discrete lag time prior to production.

Metabolizable energy (ME, MJ/kg DM) was estimated according to Menke et al. [24] as:

$$ME = 2.20 + 0.136\ GP + 0.0057\ CP\ \left({}^{g}/_{kg}\ DM\right)$$

where GP is net gas production (mL/200 mg DM) after 24 hours of incubation.

Short chain fatty acids concentration (SCFA) was calculated according to Getachew et al. [25] as:

$$SCFA\left({}^{mmol}/_{200\ mg\ DM}\right) = 0.0222\ GP - 0.00425$$

where GP is the 24 h net gas production (mL/200 mg DM).

## 2.8. Statistical Analyses

Gas production parameters were analyzed with the PROC MIXED procedure of SAS [22] in a 4 × 3 factorial experiment (i.e., 4 *Caesalpinia coriaria* extract doses and 3 fecal innocula) and the statistical model was:

$$Y_{ijk} = \mu + S_i + R_j + S_i * R_j + \varepsilon_{ijk}$$

where $Y_{ijk}$ represents every observation of the $i$th dose when incubated in the $j$th fecal inoculum, $S_i$ = the dose effect (0-, 0.6-, 1.2- and 1.8-mL), $R_j$ (j = Fecal 0, Fecal 60, or Fecal 120) is the fecal inoculum effect, $S_i{}^*R_j$ is the interaction of dose and fecal inoculum, and $\varepsilon_{ijk}$ is the experimental error.

# 3. Results

## 3.1. Gas Production, $CH_4$, CO and $H_2S$

Figs. 1-4 showed the impact of dietary inclusion of *Caesalpinia coriaria* extract (Jacq.) Wild fruit (i.e., CC) at 0-, 0.6-, 1.2- and 1.8-mL/g DM incubated on GP, $CH_4$, $H_2S$ and CO. Fig. 1 showed that the feces of the horses given 60 mL/day CC extract produced the highest ($P < .05$) gas while those given 120 mL/day for 30 days

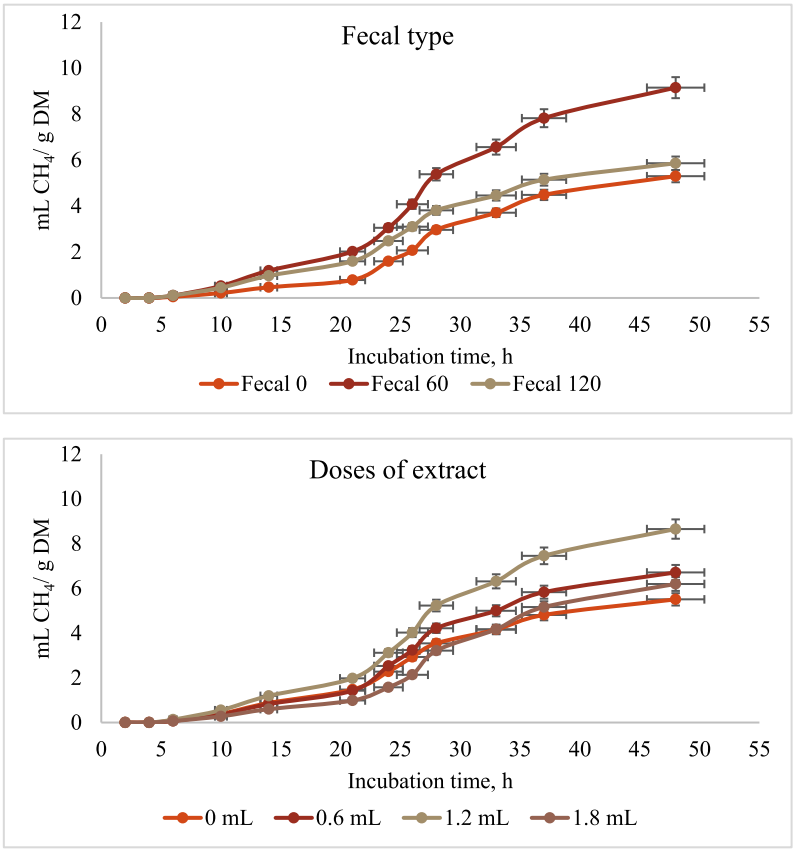


**Fig 2.** Horse methane ($CH_4$) production (mL/g dry matter (DM)) at different hours of incubation as affected by the dietary inclusion of the accuse extract of *Caesalpinia coriaria* (Jacq.) Wild fruit at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/d/horse during 30 days of the experiment.

produced the least. At 48 hours of incubation, 0 mL/g DM incubated produced the highest ($P < .05$) gas while 0.6 mL/g DM incubation of CC produced the lowest. Methane output showed that feces from horses given 60 mL/day of CC produced the highest ($P < .05$) during incubation while those that were not given CC for 30 days produced the lowest. However, dose showed that inclusion of CC at 1.2 mL/ g DM incubated produced the highest ($P < .05$) methane while 0 mL/g DM produced the lowest. All CC administered to equine or during incubation resulted in higher ($P < .05$) $CH_4$ than the control. Feces from horses that were given no CC extract produced the highest ($P < .05$) CO while those given 120 mL/day produced the lowest. Furthermore, inclusion of 1.2 mL/g DM incubated CC produced the highest ($P < .05$) CO while 0 mL/g DM produced the lowest. All CC doses produced more CO than the control. Fig. 4 showed the trend of hydrogen sulfide produced as influenced by fecal types and dosage concentration. It showed that feces of horses given 60 mL/day used for incubation produced the highest ($P < .05$) $H_2S$ while those given 0 and 120 mL/day produced the lowest. In addition, the impact of dose showed that the 0 mL/g DM incubated produced the highest ($P < .05$) while 1.8 mL/g DM produced the lowest $H_2S$.

### 3.2. Gas Production and Kinetics

The interaction of fecal type and dose at 6, 24 and 48 hours affected ($P < .05$) gas production (mL gas/g DM degraded). It showed that, in Fecal 0 horses, with 1.8 mL/g DM, produced the highest ($P < .05$) total gas, while Fecal 60 horses with 0.6 mL/g DM, increased total gas. However, the the lowest gas production was in Fecal 0 and 60 horses, at 1.2mL/g DM in 24 and 48 hours of incubation. Meanwhile at Fecal 120, 1.2 mL/g DM produced the highest ($P < .05$), while 0.6 mL/g DM produced the lowest ($P < .05$) at 24 hours of incubation. At 48 hours, 0 mL/g DM had the highest ($P < .05$) gas production while 0.6 mL/g DM, had the lowest ($P < .05$) in feces of horses given 120 mL/day CC extract. Fecal type, the dosages of extract, and their interaction had no effect on asymptotic gas production, rate of gas production, and delay before gas production begins (Lag time). Similarly, fecal type and dosage had no significant ($P > .05$) effect on the gas production at 24 and 48 hours (Table 2).

### 3.3. Methane Production and Kinetics

Table 3 showed that the feces from the horses given no CC extract produced the lowest ($P = .0014$) $CH_4$ while the feces from horses given oral CC produced more methane and Fecal 120 producedthe highest. It was also observed that all CC doses linearly ($P = .0457$) produced more $CH_4$ than the control. There was a dose-dependent increase in $CH_4$ where 1.2 mL/g DM produced the highest ($P < .05$). Horses of Fecal 60 produced the highest ($P < .05$) $CH_4$, followed by Fecal 120 while Fecal 0 produced the lowest ($P < .05$). In contrast, dose, and fecal type x dose had no impact on $CH_4$ proportions Table 4.

### 3.4. Carbon Monoxide Production and Kinetics

Table 5 showed that fecal type had a linear effect ($P < .02$) on asymptotic gas production and Lag time. Fecal type and dose interaction had a significant effect ($P = .0062$) on the rate of carbon monoxide (CO) production (/hour). Horses of Fecal 0 had the highest ($P < .05$) asymptotic CO and the longest ($P < .05$) initial delay before CO production, while Fecal 120 and Fecal 60 horses had the

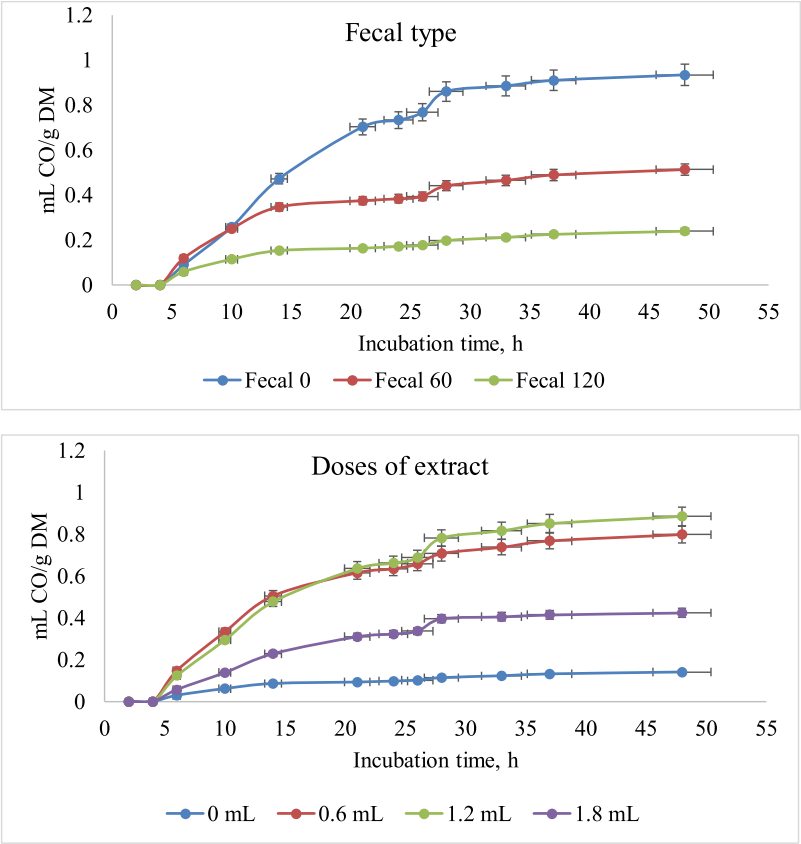


**Fig 3.** Horse carbon monoxide (CO) production (mL/g dry matter (DM)) at different hours of incubation as affected by the dietary inclusion of the accuse extract of *Caesalpinia coriaria* (Jacq.) Wild fruit at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/d/horse during 30 days of the experiment.

**Table 2**
*In vitro* gas production kinetics and total production at 6, 24 and 48 hours of the incubated and degraded diet at doses of *Caesalpinia coriaria* (Jacq.) Wild fruit accuse extract at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/d/horse during 30 days of the experiment.

| Fecal Type | Extract Dose (mL/g DM) | Gas Production Kinetics[a] | | | Gas Production (mL Gas/g DM Incubated) | | | Gas Production (mL Gas/g DM Degraded) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | b | C | Lag | 6h | 24h | 48h | 6h | 24h | 48h |
| Fecal 0 | 0 | 245.6 | 0.039 | 1.878 | 101.1 | 195 | 257.3 | 444.1 | 861.7 | 1149.2 |
| | 0.6 | 238.1 | 0.068 | 1.466 | 126.5 | 219.7 | 258 | 477.2 | 828.8 | 975 |
| | 1.2 | 262.9 | 0.04 | 5.489 | 122.5 | 210.4 | 272.5 | 457.7 | 784.4 | 1010.9 |
| | 1.8 | 327.6 | 0.037 | 3.362 | 137 | 262.7 | 340.1 | 546.9 | 1045.1 | 1353.6 |
| | Linear | 0.13 | 0.872 | 0.506 | 0.276 | 0.207 | 0.172 | 0.44 | 0.393 | 0.408 |
| | Quadratic | 0.59 | 0.829 | 0.159 | 0.9 | 0.676 | 0.598 | 0.739 | 0.365 | 0.27 |
| Fecal 60 | 0 | 319 | 0.051 | 3.564 | 147 | 259.8 | 358.4 | 609.9 | 1073.8 | 1483.9 |
| | 0.6 | 353.2 | 0.065 | 0.55 | 171.1 | 311.5 | 403.2 | 794.9 | 1448.6 | 1873.4 |
| | 1.2 | 281.1 | 0.052 | 1.692 | 117.8 | 212.9 | 294.8 | 445.3 | 804 | 1130.2 |
| | 1.8 | 332.3 | 0.089 | 2.034 | 167.6 | 299.2 | 346.9 | 631.8 | 1129.5 | 1314.7 |
| | Linear | 0.832 | 0.203 | 0.346 | 0.566 | 0.581 | 0.894 | 0.869 | 0.835 | 0.61 |
| | Quadratic | 0.425 | 0.477 | 0.427 | 0.223 | 0.293 | 0.448 | 0.154 | 0.221 | 0.358 |
| Fecal 120 | 0 | 337 | 0.029 | 4.945 | 136.1 | 246.1 | 361.8 | 613.4 | 1108.2 | 1631.1 |
| | 0.6 | 269.5 | 0.03 | 4.51 | 49.3 | 92.6 | 129.1 | 218.1 | 413.5 | 576.8 |
| | 1.2 | 292 | 0.29 | 2.415 | 170.2 | 265.2 | 336.9 | 678.6 | 1057.2 | 1343 |
| | 1.8 | 180.3 | 0.092 | 2.219 | 80.8 | 140.3 | 154.2 | 307.1 | 529.3 | 580.4 |
| | Linear | 0.049 | 0.677 | 0.11 | 0.207 | 0.225 | 0.067 | 0.11 | 0.124 | 0.034 |
| | Quadratic | 0.583 | 0.107 | 0.4 | 0.115 | 0.332 | 0.379 | 0.178 | 0.437 | 0.523 |
| SEM pooled[b] | | 37.39 | 0.0279 | 0.9892 | 22.07 | 41.54 | 49.81 | 93.7 | 178.45 | 214.78 |
| *P* value | | | | | | | | | | |
| Fecal | | | | | | | | | | |
| Linear | | 0.088 | 0.67 | 0.226 | 0.113 | 0.159 | 0.102 | 0.064 | 0.098 | 0.057 |
| Quadratic | | 0.336 | 0.149 | 0.192 | 0.089 | 0.049 | 0.054 | 0.132 | 0.074 | 0.088 |
| Dose | | | | | | | | | | |
| Linear | | 0.557 | 0.512 | 0.37 | 0.985 | 0.992 | 0.34 | 0.472 | 0.477 | 0.087 |
| Quadratic | | 0.701 | 0.11 | 0.823 | 0.633 | 0.898 | 0.966 | 0.982 | 0.581 | 0.586 |
| Fecal × Dose[a] | | 0.128 | 0.209 | 0.124 | 0.032 | 0.099 | 0.056 | 0.013 | 0.038 | 0.018 |

[a] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h).
[b] SEM standard error of the mean.

**Table 3**
*In vitro* methane production kinetics and total production at 6, 24 and 48 hours of the incubated and degraded diet at doses of *Caesalpinia coriaria* (Jacq.) Wild fruit accuse extract at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/day/horse during 30 days of the experiment.

| Fecal Type | Extract Dose (mL/g DM) | $CH_4$ Production Kinetics[a] | | | $CH_4$ Production (mL $CH_4$/g DM Incubated) | | | $CH_4$ Production (mL $CH_4$/g DM Degraded) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | b | c | Lag | 6h | 24h | 48h | 6h | 24h | 48h |
| Fecal 0 | 0 | 12.62 | 0.037 | 10.631 | 0.07 | 1.12 | 3.14 | 0.34 | 5.01 | 14.35 |
| | 0.6 | 22.32 | 0.062 | 15.166 | 0.09 | 2.53 | 6.06 | 0.31 | 9.44 | 22.78 |
| | 1.2 | 19.71 | 0.046 | 15.486 | 0.05 | 1.65 | 5.72 | 0.18 | 6.09 | 21.01 |
| | 1.8 | 18.25 | 0.039 | 18.517 | 0 | 1.08 | 6.27 | 0 | 4.43 | 25.03 |
| | Linear | 0.137 | 0.791 | 0.006 | 0.058 | 0.929 | 0.055 | 0.031 | 0.742 | 0.096 |
| | Quadratic | 0.186 | 0.352 | 0.629 | 0.698 | 0.21 | 0.424 | 0.894 | 0.382 | 0.795 |
| Fecal 60 | 0 | 20.63 | 0.045 | 11.315 | 0.11 | 2.92 | 7.57 | 0.44 | 12.13 | 31.35 |
| | 0.6 | 24.24 | 0.047 | 13.466 | 0.09 | 3.42 | 10.33 | 0.44 | 16.19 | 48.82 |
| | 1.2 | 35.46 | 0.154 | 15.551 | 0.1 | 3.42 | 10.07 | 0.37 | 13.53 | 39.46 |
| | 1.8 | 24.27 | 0.037 | 14.201 | 0.13 | 2.45 | 8.62 | 0.5 | 9.22 | 32.67 |
| | Linear | 0.496 | 0.926 | 0.326 | 0.723 | 0.482 | 0.647 | 0.873 | 0.407 | 0.903 |
| | Quadratic | 0.019 | 0.143 | 0.276 | 0.741 | 0.224 | 0.332 | 0.739 | 0.351 | 0.436 |
| Fecal 120 | 0 | 15.812 | 0.057 | 11.629 | 0.07 | 2.79 | 5.82 | 0.3 | 12.54 | 26.21 |
| | 0.6 | 22.062 | 0.052 | 10.988 | 0.04 | 1.65 | 3.75 | 0.2 | 7.32 | 16.64 |
| | 1.2 | 29.023 | 0.042 | 9.612 | 0.24 | 4.29 | 10.17 | 0.98 | 17.11 | 40.55 |
| | 1.8 | 22.615 | 0.043 | 14.577 | 0.06 | 1.2 | 3.7 | 0.24 | 4.51 | 14.03 |
| | Linear | 0.172 | 0.07 | 0.108 | 0.966 | 0.248 | 0.518 | 0.848 | 0.173 | 0.38 |
| | Quadratic | 0.037 | 0.207 | 0.038 | 0.016 | 0.071 | 0.081 | 0.019 | 0.102 | 0.109 |
| SEM pooled[a] | | 2.712 | 0.0136 | 1.3158 | 0.034 | 0.497 | 1.466 | 0.143 | 2.11 | 6.208 |
| *P* value | | | | | | | | | | |
| Fecal | | | | | | | | | | |
| Linear[b] | | 0.001 | 0.305 | 0.245 | 0.097 | 0.003 | 0.003 | 0.1 | 0.002 | 0.003 |
| Quadratic | | 0.921 | 0.629 | 0.012 | 0.354 | 0.684 | 0.193 | 0.379 | 0.603 | 0.263 |
| Dose | | | | | | | | | | |
| Linear | | 0.046 | 0.811 | 0.002 | 0.654 | 0.174 | 0.617 | 0.487 | 0.091 | 0.992 |
| Quadratic | | 0 | 0.121 | 0.949 | 0.084 | 0.011 | 0.026 | 0.144 | 0.033 | 0.069 |
| Fecal × Dose | | 0.489 | 0.417 | 0.206 | 0.094 | 0.146 | 0.269 | 0.165 | 0.181 | 0.205 |

[a] *b* is the asymptotic $CH_4$ production (mL/g DM); *c* is the rate of $CH_4$ production (/h); *Lag* is the initial delay before $CH_4$ production begins (h).
[b] SEM standard error of the mean.

**Table 4**
*In vitro* methane proportions at 6, 24 and 48 hours of the incubated and degraded diet at doses of *Caesalpinia coriaria* (Jacq.) Wild fruit accuse extract at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/day/horse during 30 days of the experiment.

| Fecal Type | Extract Dose (mL/g DM) | mL $CH_4$/ 100 mL Gas | | | mg $CH_4$/mL Gas | | | g $CH_4$/ kg DM | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 6h | 24h | 48h | 6h | 24h | 48h | 6h | 24h | 48h |
| Fecal 0 | 0 | 0.88 | 6.3 | 12.89 | 0.35 | 2.52 | 5.15 | 0.34 | 5.22 | 14.59 |
| | 0.6 | 0.92 | 12.19 | 23.94 | 0.37 | 4.88 | 9.58 | 0.4 | 11.74 | 28.19 |
| | 1.2 | 0.38 | 7.92 | 21 | 0.15 | 3.17 | 8.4 | 0.23 | 7.7 | 26.59 |
| | 1.8 | 0 | 4.13 | 18.46 | 0 | 1.65 | 7.38 | 0 | 5.02 | 29.14 |
| | Linear | 0.119 | 0.319 | 0.137 | 0.12 | 0.32 | 0.14 | 0.06 | 0.93 | 0.05 |
| | Quadratic | 0.889 | 0.166 | 0.105 | 0.889 | 0.166 | 0.105 | 0.696 | 0.209 | 0.424 |
| Fecal 60 | 0 | 0.75 | 11.53 | 21.41 | 0.3 | 4.61 | 8.56 | 0.49 | 13.6 | 35.2 |
| | 0.6 | 0.46 | 10.84 | 25.34 | 0.18 | 4.34 | 10.14 | 0.4 | 15.91 | 48.02 |
| | 1.2 | 0.75 | 21.85 | 37.23 | 0.3 | 8.74 | 14.89 | 0.45 | 15.91 | 46.83 |
| | 1.8 | 0.79 | 8.22 | 24.72 | 0.32 | 3.29 | 9.89 | 0.63 | 11.39 | 40.09 |
| | Linear | 0.918 | 0.614 | 0.56 | 0.918 | 0.614 | 0.56 | 0.722 | 0.482 | 0.647 |
| | Quadratic | 0.952 | 0.06 | 0.017 | 0.952 | 0.06 | 0.017 | 0.742 | 0.224 | 0.332 |
| Fecal 120 | 0 | 0.5 | 11.28 | 16.03 | 0.2 | 4.51 | 6.41 | 0.31 | 12.95 | 27.05 |
| | 0.6 | 1.08 | 14.83 | 22.67 | 0.43 | 5.93 | 9.07 | 0.21 | 7.65 | 17.42 |
| | 1.2 | 1.54 | 16.36 | 29.82 | 0.62 | 6.55 | 11.93 | 1.13 | 19.94 | 47.28 |
| | 1.8 | 0.67 | 8.64 | 23.22 | 0.27 | 3.45 | 9.29 | 0.29 | 5.57 | 17.21 |
| | Linear | 0.77 | 0.245 | 0.166 | 0.77 | 0.245 | 0.166 | 0.967 | 0.248 | 0.518 |
| | Quadratic | 0.08 | 0.008 | 0.037 | 0.08 | 0.008 | 0.037 | 0.016 | 0.071 | 0.081 |
| SEM pooled[a] | | 0.271 | 1.81 | 2.768 | 0.108 | 0.724 | 1.107 | 0.159 | 2.31 | 6.816 |
| *P* value | | | | | | | | | | |
| Fecal | | | | | | | | | | |
| Linear | | 0.554 | 0.012 | 0.002 | 0.554 | 0.012 | 0.002 | 0.097 | 0.003 | 0.003 |
| Quadratic | | 0.126 | 0.18 | 0.926 | 0.126 | 0.18 | 0.926 | 0.356 | 0.684 | 0.193 |
| Dose | | | | | | | | | | |
| Linear | | 0.436 | 0.254 | 0.055 | 0.436 | 0.254 | 0.055 | 0.655 | 0.174 | 0.617 |
| Quadratic | | 0.242 | 0.002 | 0 | 0.242 | 0.002 | 0 | 0.083 | 0.011 | 0.026 |
| Fecal × Dose | | 0.243 | 0.312 | 0.454 | 0.243 | 0.312 | 0.454 | 0.094 | 0.146 | 0.269 |

[a] SEM standard error of the mean.

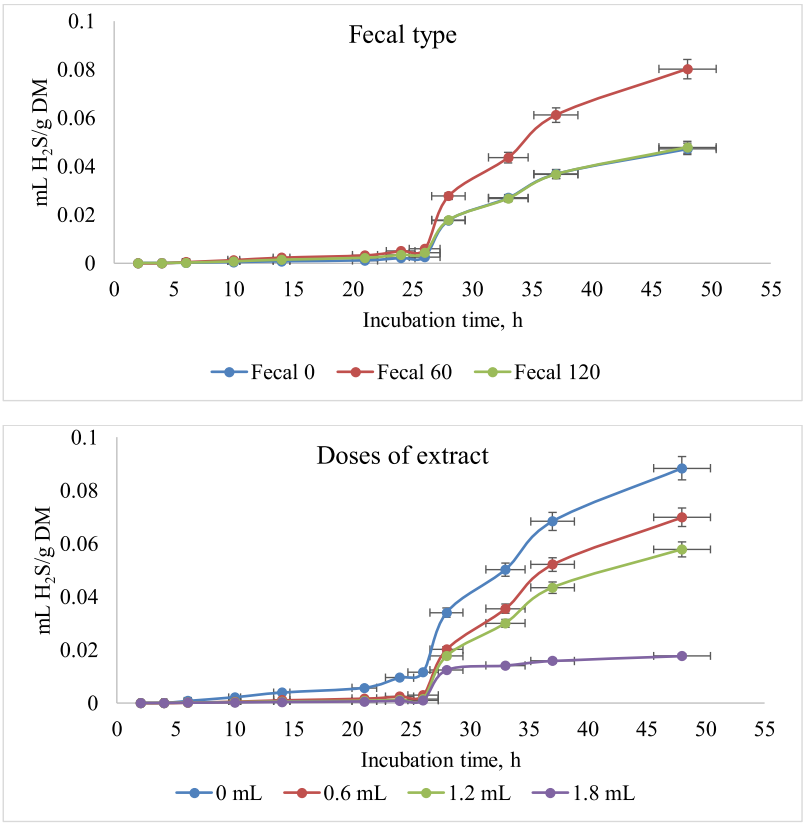


**Fig 4.** Horse hydrogen sulfide ($H_2S$) production (mL/g dry matter (DM)) at different hours of incubation as affected by the dietary inclusion of the accuse extract of *Caesalpinia coriaria* (Jacq.) Wild fruit at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/d/horse during 30 days of the experiment.

**Table 5**
*In vitro* carbon monoxide (CO) production kinetics and total production at 6, 24 and 48 hours of the incubated and degraded diet at doses of *Caesalpinia coriaria* (Jacq.) Wild fruit accuse extract at 0-, 0.6-, 1.2- and 1.8- mL/ g DM incubated with fecal content of horses that received the same extract at 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/day/horse during 30 days of the experiment.

| Fecal Type | Extract Dose (mL/g DM) | CO Production Kinetics[a] | | | CO Production (mL CO /g DM Incubated) | | | CO Production (mL CO /g DM Degraded) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | b (ppm) | c | Lag | 6h | 24h | 48h | 6h | 24h | 48h |
| Fecal 0 | 0 | 45.4 | 0.064 | 8.697 | 0.001 | 0.004 | 0.012 | 0.004 | 0.019 | 0.052 |
| | 0.6 | 4226 | 0.805 | 22.983 | 0.164 | 0.989 | 1.183 | 0.668 | 3.828 | 4.582 |
| | 1.2 | 3268.8 | 0.072 | 8.305 | 0.036 | 1.004 | 1.316 | 0.116 | 3.664 | 4.78 |
| | 1.8 | 2421.4 | 0.783 | 13.021 | 0.161 | 0.933 | 1.226 | 0.631 | 3.576 | 4.698 |
| | Linear | 0.052 | 0.03 | 0.641 | 0.144 | 0.044 | 0.049 | 0.158 | 0.044 | 0.046 |
| | Quadratic | 0.054 | 0.176 | 0.75 | 0.612 | 0.152 | 0.162 | 0.579 | 0.186 | 0.197 |
| Fecal 60 | 0 | 342.1 | 0.293 | 3.739 | 0.03 | 0.102 | 0.143 | 0.122 | 0.411 | 0.575 |
| | 0.6 | 2166.1 | 0.773 | 5.242 | 0.215 | 0.696 | 0.906 | 1.031 | 3.331 | 4.334 |
| | 1.2 | 3091.3 | 0.8 | 5.275 | 0.223 | 0.71 | 0.973 | 0.837 | 2.661 | 3.688 |
| | 1.8 | 86.9 | 0.07 | 2.566 | 0.007 | 0.024 | 0.031 | 0.025 | 0.088 | 0.114 |
| | Linear | 0.682 | 0.177 | 0.152 | 0.779 | 0.777 | 0.738 | 0.79 | 0.787 | 0.752 |
| | Quadratic | 0.001 | 0.002 | 0.011 | 0.02 | 0.028 | 0.013 | 0.037 | 0.043 | 0.025 |
| Fecal 120 | 0 | 650.7 | 0.29 | 2.587 | 0.056 | 0.186 | 0.268 | 0.252 | 0.835 | 1.201 |
| | 0.6 | 2568 | 0.542 | 5.554 | 0.063 | 0.217 | 0.306 | 0.278 | 0.961 | 1.353 |
| | 1.2 | 1226.7 | 0.693 | 5.174 | 0.113 | 0.271 | 0.367 | 0.452 | 1.081 | 1.463 |
| | 1.8 | 123.4 | 0.178 | 4.178 | 0.003 | 0.011 | 0.016 | 0.008 | 0.039 | 0.055 |
| | Linear | 0.491 | 0.683 | 0.196 | 0.428 | 0.366 | 0.358 | 0.409 | 0.349 | 0.341 |
| | Quadratic | 0.221 | 0.079 | 0.104 | 0.172 | 0.308 | 0.346 | 0.218 | 0.38 | 0.42 |
| SEM pooled[a] | | 477.53 | 0.1298 | 2.1719 | 0.0453 | 0.1508 | 0.2025 | 0.1863 | 0.6205 | 0.8003 |
| *P* value | | | | | | | | | | |
| Fecal | | | | | | | | | | |
| Linear | | 0.015 | 0.655 | 0.002 | 0.496 | 0.025 | 0.039 | 0.401 | 0.064 | 0.093 |
| Quadratic | | 0.029 | 0.757 | 0.066 | 0.214 | 0.006 | 0.008 | 0.24 | 0.009 | 0.011 |
| Dose | | | | | | | | | | |
| Linear | | 0.268 | 0.356 | 0.604 | 0.571 | 0.195 | 0.215 | 0.64 | 0.245 | 0.267 |
| Quadratic | | <0.0001 | 0.051 | 0.863 | 0.062 | 0.005 | 0.005 | 0.104 | 0.011 | 0.009 |
| Fecal × Dose[b] | | 0.062 | 0.006 | 0.439 | 0.112 | 0.109 | 0.109 | 0.121 | 0.11 | 0.103 |

[a] *b* is the asymptotic Carbon monoxide (CO) production (ppm); *c* is the rate of Carbon monoxide (CO) production (/h); *Lag* is the initial delay before Carbon monoxide (CO) production begins (h).
[b] SEM standard error of the mean.

**Table 6**
*In vitro* hydrogen sulfide ($H_2S$) production kinetics, total production at 6, 24 and 48 hours of the incubated and degraded diet at doses of *Caesalpinia coriaria* (Jacq.) Wild fruit accuse extract at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/day/horse during 30 days of the experiment.

| Fecal Type | Extract Dose (mL/g DM) | $H_2S$ Production Kinetics[a] | | | $H_2S$ Production (mL/g DM Incubated)[b] | | $H_2S$ Production (mL/g DM Degraded)[b] | |
|---|---|---|---|---|---|---|---|---|
| | | b (ppm) | c | Lag | 24h | 48h | 24h | 48h |
| Fecal 0 | 0 | 168.8 | 0.176 | 4.237 | 0.005 | 0.045 | 0.024 | 0.202 |
| | 0.6 | 230.5 | 0.053 | 5.467 | 0.001 | 0.065 | 0.005 | 0.242 |
| | 1.2 | 218.2 | 0.049 | 7.015 | 0.001 | 0.058 | 0.003 | 0.217 |
| | 1.8 | 57.9 | 0.393 | 5.609 | 0.001 | 0.021 | 0.003 | 0.082 |
| | Linear | 0.04 | 0.134 | 0.34 | 0.01 | 0.272 | 0.001 | 0.15 |
| | Quadratic | 0.028 | 0.07 | 0.112 | 0.059 | 0.193 | 0.023 | 0.284 |
| Fecal 60 | 0 | 389.6 | 0.035 | 2.315 | 0.012 | 0.112 | 0.05 | 0.469 |
| | 0.6 | 337.4 | 0.042 | 5.427 | 0.006 | 0.127 | 0.025 | 0.582 |
| | 1.2 | 188.2 | 0.167 | 5.383 | 0.002 | 0.057 | 0.006 | 0.22 |
| | 1.8 | 72.2 | 0.571 | 6.251 | 0.001 | 0.025 | 0.004 | 0.097 |
| | Linear | 0.004 | 0.007 | <0.0001 | <0.0001 | <0.0001 | <.0001 | <0.0001 |
| | Quadratic | 0.546 | 0.156 | 0.019 | 0.004 | 0.292 | 0.006 | 0.055 |
| Fecal 120 | 0 | 333.7 | 0.062 | 3.06 | 0.011 | 0.108 | 0.05 | 0.491 |
| | 0.6 | 194.8 | 0.263 | 6.225 | 0 | 0.018 | 0.003 | 0.082 |
| | 1.2 | 197.5 | 0.164 | 6.297 | 0.001 | 0.059 | 0.005 | 0.235 |
| | 1.8 | 46.9 | 0.327 | 6.658 | 0 | 0.007 | 0.002 | 0.025 |
| | Linear | 0.023 | 0.2 | 0.009 | <0.0001 | 0.003 | <0.0001 | 0.002 |
| | Quadratic | 0.937 | 0.856 | 0.155 | 0.004 | 0.956 | 0.002 | 0.807 |
| SEM pooled[c] | | 45.08 | 0.0768 | 0.6106 | 0.0006 | 0.0113 | 0.0025 | 0.0425 |
| *P* value | | | | | | | | |
| Fecal | | | | | | | | |
| Linear | | 0.059 | 0.625 | 0.162 | <.0001 | 0.0022 | <.0001 | 0.004 |
| Quadratic | | 0.672 | 0.778 | 0.441 | 0.517 | 0.072 | 0.968 | 0.108 |
| Dose | | | | | | | | |
| Linear | | <0.0001 | 0.0005 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Quadratic | | 0.562 | 0.077 | 0.006 | <0.0001 | 0.626 | <0.0001 | 0.924 |
| Fecal × Dose | | 0.267 | 0.313 | 0.522 | 0.006 | 0.001 | 0.003 | 0.002 |

[a] *b* is the asymptotic Hydrogen sulfide ($H_2S$) production (ppm); *c* is the rate of Hydrogen sulfide ($H_2S$) production (/h); *Lag* is the initial delay before Hydrogen sulfide ($H_2S$) production begins (h).
[b] Production of $H_2S$ at 6 hours (mL/g DM incubated and degraded) was zero.
[c] SEM standard error of the mean.

lowest ($P < .05$), respectively. The fecal x dose showed that under Fecal 0, 0.6 mL/g DM, had the highest ($P < .05$) rate of CO production during incubation, while 0 mL/g DM had the lowest ($P < .05$) rate. In Fecal 60 and 120 horses, 1.2 mL/g DM, had the highest ($P < .05$) rate of CO production, while it was the lowest ($P < .05$) with 1.8 mL/g DM. In contrast, in the feces of horses given *Caesalpinia coriaria*, dose of CC extract, or feces x dose interaction had no significant ($P > .05$) impact on CO production.

### 3.5. Hydrogen Sulfide Kinetics and Fecal Fermentation Profile

The $H_2S$ showed that feces of equine given 60 mL/day CC produced the highest ($P < .05$), while Fecal 0 and Fecal 120 horses were similar. Fecal type x dose showed that 0 mL/g DM produced the highest ($P < .05$) $H_2S$, while 1.8 mL/g DM produced the lowest ($P < .05$). (Table 6).

The impact of *Caesalpinia coriaria* given as extract to horses on fecal fermentation profile and $CH_4$ conversion efficiency are shown in Table 7. The result showed that in both Fecal 0 and 120 samples, the dose of 1.2 mL/g DM during incubation had the highest ($P = .0483$) pH, while 0 mL/g DM had the lowest ($P < .05$) . Furthermore, in feces obtained from horses given 60 mL/day (i.e., Fecal 60) of CC for 30 days, 0 mL/g DM had the highest ($P < .05$) pH, while 0.6 mL/g DM had the lowest ($P < .05$) value when used to incubate diet. Fecal types linearly ($P < .02$) affected $CH_4$ conversion efficiency. The result showed that fecal type 0 (Fecal 0) was more efficient and produced less $CH_4$ for every unit of ME, OM, and SCFA, while Fecal 60 was the least efficient (Table 7).

## 4. Discussion

### 4.1. Total Gas

Gas production and Lag time dynamics reveal the impact of additives (microbial, phytogenic, synthetics, enzymes) on diet and how animals could benefit *in vivo*. Furthermore, it depicts nutrient utilization, energy, and digestibility. Synergistic action occurred between fecal types and doses of CC which aided the digestibility of diet and increased gas production. Except for Fecal 120, in all other fecal types, it was observed that CC administration increased feed digestibility than the control. The increase in production may be due to the stimulatory activity of phenol on hindgut microbes to digest the highly fibrous diet at 24 h [26,27]. However, looking at the gas production per gram of incubated diet, it indicates that in Fecal 0 and Fecal 60 with higher dosage of CC, during incubation, led to a lower gas production compared to no CC addition at 48 hours. Could this be that equine microbes are quite sensitive to the prolonged use of CC at higher dose, and this question is further reinforced by the gas production observed in Fecal 120 horses where higher CC supplementations (Fecal 60 and 120) and all extracts (0.6-, 1.2- and 1.8- mL) produced lower gas compared to 0 mL. This suggest that prolonged use of CC in equine may be too toxic/sensitive for equine microbes due to the tannin which exhibited antimicrobial activities at higher dose [28]. Furthermore, it showed that at modest CC dose, it can stimulate the microbial activity to aid digestion. It also depicts that CC at medium dose can help to derive more nutrient for every gram of feed degraded

**Table 7**
*In vitro* fermentation profile and methane conversion efficiency to short chain fatty acids ($CH_4$: SCFA at 24 hours, mmol/mmol), metabolizable energy ($CH_4$: ME (g/MJ)), and organic matter ($CH_4$:OM (mL/g)) of the dietary inclusion of different doses of *Caesalpinia coriaria* (Jacq.) Wild fruit accuse extract at 0-, 0.6-, 1.2- and 1.8- mL/g DM incubated with fecal content of horses that received the same extract of 0- (Fecal 0), 60- (Fecal 60) and 120- (Fecal 120) mL/day/horse during 30 days of the experiment.

| Fecal type | Extract dose (mL/g DM) | Fecal Fermentation Profile | | | | $CH_4$: ME (g/MJ) | Methane Conversion Efficiency | |
|---|---|---|---|---|---|---|---|---|
| | | pH | DMD% | SCFA, mmol/g DM | ME, MJ/kg DM 24h | | $CH_4$:OM (mL/g) | $CH_4$: SCFA at 24h (mmol/mmol) |
| Fecal 0 | 0 | 6.22 | 44.79 | 4.31 | 6.19 | 0.85 | 1.25 | 8.27 |
| | 0.6 | 6.31 | 53.11 | 4.86 | 6.47 | 1.79 | 2.81 | 15.99 |
| | 1.2 | 6.58 | 55.1 | 4.65 | 6.37 | 1.2 | 1.84 | 10.38 |
| | 1.8 | 6.39 | 51.07 | 5.81 | 6.96 | 0.72 | 1.2 | 5.4 |
| | Linear | 0.002 | 0.255 | 0.207 | 0.207 | 0.619 | 0.929 | 0.319 |
| | Quadratic | <0.0001 | 0.145 | 0.676 | 0.676 | 0.097 | 0.21 | 0.168 |
| Fecal 60 | 0 | 6.48 | 47.47 | 5.75 | 6.93 | 1.92 | 3.25 | 15.11 |
| | 0.6 | 5.9 | 43.64 | 6.9 | 7.52 | 2.1 | 3.8 | 14.19 |
| | 1.2 | 6.18 | 50.86 | 4.71 | 6.4 | 2.56 | 3.8 | 28.77 |
| | 1.8 | 6.28 | 53.04 | 6.62 | 7.38 | 1.54 | 2.72 | 10.76 |
| | Linear | 0.061 | 0.1849 | 0.5805 | 0.58 | 0.334 | 0.482 | 0.617 |
| | Quadratic | 0.042 | 0.859 | 0.294 | 0.293 | 0.03 | 0.224 | 0.061 |
| Fecal 120 | 0 | 5.78 | 44.24 | 5.44 | 6.77 | 1.89 | 3.1 | 14.78 |
| | 0.6 | 5.99 | 42.75 | 2.04 | 5.02 | 1.2 | 1.83 | 20.14 |
| | 1.2 | 6.19 | 50.15 | 5.87 | 6.99 | 2.79 | 4.76 | 21.44 |
| | 1.8 | 6.07 | 54.69 | 3.09 | 5.57 | 0.97 | 1.33 | 11.36 |
| | Linear | 0.334 | 0.014 | 0.225 | 0.225 | 0.269 | 0.248 | 0.222 |
| | Quadratic | 0.315 | 0.82 | 0.332 | 0.332 | 0.075 | 0.071 | 0.006 |
| SEM pooled[b] | | 0.084 | 2.611 | 0.922 | 0.474 | 0.288 | 0.552 | 2.355 |
| *P* value | | | | | | | | |
| Fecal | | | | | | | | |
| Linear | | 0.07 | 0.289 | 0.1589 | 0.159 | 0.002 | 0.003 | 0.012 |
| Quadratic | | 0.001 | 0.296 | 0.049 | 0.049 | 0.572 | 0.684 | 0.16 |
| Dose | | | | | | | | |
| Linear | | 0.389 | 0.005 | 0.992 | 0.991 | 0.123 | 0.174 | 0.257 |
| Quadratic[a] | | 0.205 | 0.189 | 0.9 | 0.898 | 0.002 | 0.011 | 0.002 |
| Fecal × Dose | | 0.048 | 0.258 | 0.099 | 0.099 | 0.16 | 0.146 | 0.3 |

[a] SCFA is the short chain fatty acids (mmol/g DM); DMD is the *in vitro* dry matter digestibility (%); ME is the metabolizable energy (MJ/kg DM).
[b] SEM standard error of the mean.

thereby reducing the methane output per horse *in vivo* and shift it to outside the body.

### 4.2. Methane

Compared to ruminants, $CH_4$ production in equine is quite low due to various factors such as shorter retention time for holding feed, variation in the structural design of the gut anatomy, and lower $CH_4$ aiding microbes [1,11,29]. Increasing equine population could enhance $CH_4$ output both directly and indirectly. It was observed that all measurement and conversion efficiency showed that Fecal 0 horses produced the least $CH_4$ while horses of Fecal 60 produced the highest. It is important to understand that as methanogenesis is the form of $H_2$ sink in ruminants, acetogenesis is the $H_2$ sink in equine because they do not possess eructation and flatulence frequency like ruminants [1,11]. While it is established that acetogens are the $H_2$ sink in equine, their efficacy of sinking $H_2$ compared to methanogens is in the partial pressure of $H_2$ gas on biofilm. With $CH_4$ having lower threshold (6-120 ppm) compared to acetogens (430–4660 ppm), it gives methanogens the advantage of forming potent $CH_4$ compared to the $H_2$ sinking by acetogens [1,30]. In view of this, the lower $CH_4$ in Fecal 0 and Fecal 120 horses may be due to the lower $H_2S$ produced from both groups. This suggest that based on the ability of methanogens to form $CH_4$ faster than acetogens, the increasing $H_2S$ led to enhanced activity of methanogens than the acetogen's ability to sink $H_2S$. The lower $CH_4$ in Fecal 120 horses also suggest that high dose of CC in equine (120 mL/day) reduced the activities of certain $H_2$ producing microbes and methanogens. This showed that CC in the diet had negative effect on $CH_4$ production. The higher $CH_4$ in Fecal 60 horses may be due to the enhanced dietary fiber digestion which aided methanogen's activity through the $H_2$ exchange relationship with protozoa. As it could be observed, the highest gas production for degraded DM occurred at 48 h in Fecal 60 when 0.6 mL/g DM was given.

### 4.3. Hydrogen Sulfide and Carbon Monoxide

Oxidative stress is caused by an imbalance between oxidants and antioxidants. The availability of CO and $H_2S$ is to protect the gut against inflammation as well as serve as antioxidant enzyme [5]. In this study, increased $H_2S$ production in Fecal 60 horses, as well as under 0 mL dose of CC extract, suggest that a moderate quantity in equine has the potential to exhibit antioxidative activity in the hindgut which could reduce the case of disease incidence. Neal [31] reported that due to the association of dietary fiber and reduced disease incidence, the antioxidant role played by the molecular hydrogen in tissues is an important factor and it reduces the role of potentially damaging species and their involvement in diseases causation. Similarly, Corilagin a component isolated from CC exhibited antioxidant, and anti-inflammatory activity [32,33]. This suggest that CC in equine diet at a minimal input can improve fiber digestion and at the same time perform antioxidant role. However, CO production in the fecal type decreased with increasing CC horse feed supplementation. It was earlier stated that acetogenesis is the main $H_2$ sink in hindgut fermenters compared to methanogenesis which is used to produce acetate. The lower $CH_4$ in Fecal 0 horses may be due to acetogenesis processes. The acetogens uses anaerobic carbon monoxide dehydrogenase enzymes alongside acetyl-CoA synthetase to catalyze the reduction of $CO_2$ to CO through the carbonyl branch of the acetyl-CoA pathway [34,35]. Therefore, it can be suggested during acetogenesis, acetogens were able to use $CO_2$ leading to increased CO which commensurate with lower $CH_4$.

### 4.4. Fermentation Profile

Hindgut pH influences the movement of VFA across the epithelium [36]., and the pH of equines is about 6.0 and it is optimal for fiber degrading microbes [37]. However, it was proposed that the optimal pH for appropriate microbial fermentation was 6.5 [38]. It was observed that there is no trend of the pH in this study. Notwithstanding, the group that produced the highest gas per g DM degraded throughout this study was Fecal 60 horses, at 0.6 mL/g DM and it had pH 5.9 which suggest that it could be the optimal pH for digestion of fibrous diet when CC is added to the diet.

## 5. Conclusion

Feed supplementation of *Caesalpinia coriaria* fruit (i.e., CC) extract, and/or dose during incubation, in equine, increased $CH_4$ production. It was also observed that due to lower methane and higher CO in Fecal 0 horses, acetogenesis is the likely mechanism of lower $CH_4$ output. Similarly, the highest biogas production at 48 hours occurred in Fecal 60 with 0.6 mL/g DM of CC extract. Thus, based on this, it is recommended to use the daily CC extract supplementation at 60 mL/day/horse with or without 0.6 mL/g DM CC extract.

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